C-reactive protein testing to discriminate bacterial from viral pathogens among febrile patients in Southeast Asia and its impact on antibiotic prescribing in primary care

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ABSTRACT

In an era marked by the worldwide decline of malaria, fever remains the most frequent reason for seeking health care in the tropics. Non-malarial pathogens are mainly studied among hospitalised patients, while evidence in primary care is scarce. In addition to the limited evidence on the local epidemiology, prescribers routinely face difficulty in identifying patients who really need an antibiotic. This translates into high antibiotic prescription, fuelling the worldwide spread of antimicrobial resistance. C-reactive protein (CRP) is one of the most studied host-response biomarkers for guiding the management of acutely febrile patients, although evidence mainly originates from hospitals in high-income countries, implying that findings may not be applicable in primary care in low-to-middle-income countries (LMICs). My thesis aims to explore the bacteria and viruses driving acute fever in primary care in Southeast Asia, as well as the potential utility of CRP in identifying patients who need an antibiotic. To this end, we designed an individually randomised controlled trial of CRP-guided treatment in all febrile children and adults, 'the CRP Study'. We also collected blood and respiratory specimens to ascertain the causes of fever in these patients. We found a significant antibiotic reduction of 7.8 percentage-points using CRP at a 40 mg/L threshold compared to routine practice, without differences in clinical outcome. Routine prescription levels were lower than prior to study implementation, suggesting that future studies should adopt a cluster rather than individual randomised design. Influenza virus type A, dengue virus and respiratory syncytial virus were the key pathogens among our primary care patients, although ascertaining causality was challenging due to lack of control group, especially in nasopharyngeal swabs. Most bacterial infections showed low inflammatory levels and recovered without antibiotics, questioning the interpretation of microbiological findings among non-severe patients. CRP performance in distinguishing bacterial from viral pathogens was limited, suggesting that CRP may rather discriminate self-limiting from serious infections. We also evaluated the performance of CRP in a cohort of febrile patients in Tanzania. Here CRP showed high sensitivity for detecting bacterial blood stream infection, indicating that prospective evaluations on its effect on antibiotic prescriptions could be envisaged. The detection of bacterial zoonotic pathogens was of lower accuracy, warranting further evaluations for various levels of healthcare settings and age categories.

ATTRIBUTIONS

This thesis is the result of my own work and has not been submitted to any other university. Nevertheless, all this work was only possible through a collective effort including colleague researchers, doctors, nurses, laboratory technicians, and members of the Clinical Trial Support Group (CTSG) from the Mahidol-Oxford Tropical Medicine Research Unit (MORU) network, as well as external collaborators. Each step of my thesis required specific competences beyond my own skill set, and I would like to define the limitations of my personal contribution to this thesis and attribute the roles played by the various collaborators.

The CRP Study

- **Study approval and procedures**
    - **Attribution to collaborators**

The CTSG submitted our protocol and case report form (CRF) to different ethics committees: in Thailand, the Faculty of Tropical Medicine at Mahidol University, and the Chiang Rai local Public Health Office; in Myanmar, the Department of Medical Research. The coordination with the various ethics committees was ensured by Noppawan Lunjarungsimalert, Salwaluk Panapipat in Thailand, and by Myo Maung Maung Swe in Myanmar.

Part of the study procedures including study staff training for good clinical practice (GCP), training for consenting and assenting study participants and all study steps were provided in local language by the CTSG and Myo Maung Maung Swe in Myanmar.

The data management component was organised by CTSG, including the dataset and variable design, as well as identification and emission of queries for data inconsistencies, to be checked by the study staff onsite. In particular, Bencharat Thongpunchang in Bangkok and Myo Min in Myanmar were our data manager in Bangkok, in charge of the dataset and variable verification.

- **My own contribution**

I originally drafted and designed the first version of the CRP Study protocol. This included a literature review summary, formulation of the main gaps on causes of fever in primary care and evidence on C-reactive protein (CRP) in Southeast Asian primary care. This original protocol also included the research strategy with corresponding objectives and methods, including population and setting selection, as well as sample size determination.

During the CRP Study preparation, I actively participated in the design and facilitation of all training sessions. I was the one who designed the CRF under the supervision of Naomi Waithira, head of the data management department at CTSG. On site, I was involved in the supervision of the protocol compliance, CRF completion and patient follow-up, although this role was limited to *ad hoc* visits while most of the field coordination was ensured by local study coordinators, namely Rachel Greer and Jeroen Bok in Thailand, and Myo Maung Maung Swe and Joshua Cohen in Myanmar.

In terms of data management, my role was to identify the variables to be verified, including the definition of realistic ranges for numerical variables (e.g. blood pressure) and participant's answers consistency by cross checking different variables.

- **Sample management**
  - **Attribution to collaborators**

The types of blood and urine tubes as well as nasopharyngeal (NP) swabs were selected by our study laboratory focal point, Janjira Thaipadungpanit, senior microbiologist at MORU, Bangkok, Thailand. This technical support included site visits in Chiang Rai and Hlaing Tha Yar with local laboratory staff training for sample management. The supervision and coordination with the local laboratory members were also ensured by Janjira Thaipadungpanit. The local laboratory members in charge of the sample management onsite were Nattapon Pinthong and Areerat Thaiprakhong, laboratory technicians in Thailand, and Kyaw Soe in Myanmar.

CRP was tested onsite using a point-of-care reader (Nycocard Reader II, Axis-Shield, Norway), and the training was led by Ampai Tanganuchitcharnchai, laboratory technician, in local language. Dried blood spots (DBS) were collected from capillary blood among children under 5-year-old in

Myanmar due to ethics committee decision, in order to limit the volume sampled in this population. The standard operating procedure (SOP) for DBS was designed by Pieter Smit (Appendix II), research microbiologist. Capillary blood extraction from DBS was carried out in MORU, Bangkok, by Janjira Thaipadungpanit.

- **My own contribution**

My role was to design the SOPs for blood specimens and NP swabs collection, transport and storage. I also ensured technical training sessions for NP swabs and capillary blood specimen collection, as well as tube labelling and DBS storage (Appendix I).

- **Sample analysis**
  - **Attribution to collaborators**

Venous blood and NP swabs specimens were shipped at -80˚C to MORU for extraction and analysis. The deoxyribonucleic acid (DNA) extraction was performed by Janjira Thaipadungpanit and Prapaporn Srilohasin, microbiologists. Concerning the aetiological investigations, Pieter Smit was in charge of the viral analysis both on capillary and venous blood specimens using a singleplex polymerase chain reaction (PCR) for dengue, chikungunya and zika viruses, while Janjira Thaipadungpanit was in charge of the bacterial singleplex PCR for *Rickettsia* genus, *Orientia tsutsugamushi* and *Leptospira* spp. The PCR 16S was carried out at MORU and positives were secondarily tested for DNA sequencing, and this was also managed by Janjira Thaipadungpanit. The Taqman Array Card (TAC) assay and the bacterial singleplex PCR on blood specimens were managed by Janjira Thaipadungpanit.

Furthermore, my thesis included serological methods: we measured the anti-haemolysin coregulated protein 1 (Hcp1) among our patients, as a potential marker of exposure to *Burkholderia pseudomallei*. This serological method relied on enzyme-linked immunosorbent assay (ELISA), and was designed and carried out by Narisara Chantratita and her team from Mahidol University. We also measured antibody titre for *Leptospira* spp., *O. tsutsugamushi*, typhus group and spotted fever group using ELISA and immunofluorescent antibody (IFA) test. This was managed by Ampai Tanganuchitcharnchai. Lastly, we also tested all *Leptospira* ELISA-positives using the microscopic

agglutination test (MAT), and this analysis was carried out at the National Institute for Animal Health (NIAH) in Bangkok, Thailand, by Duangjai Suwancharoen, senior microbiologist.

- **My own contribution**

My participation to the laboratory side was limited to the TAC assay for the NP swabs, including specimen processing, analysis and interpretation. Regarding the ELISA and IFA, my responsibility was restricted to the statistical analysis and report redaction.

The Tanzanian Study

My participation consisted in designing and performing the data analysis on the performance of CRP in detecting bacterial infections, and report redaction. I was not involved in the original study design, protocol writing, study implementation, sample collection nor laboratory analysis.

ACKNOWLEDGMENTS

The decision to start a DPhil in science was unexpected, as I never imagined being a scientist. My perception of science was restricted to a world ruled by mathematics and statistics, and I had never imagined myself fitting in such an environment.

I really would like to thank Yoel Lubell, my thesis supervisor, for his vision and comprehension of the challenges faced by health workers in the management of fever in a resource restricted environment. My time in Asia has been the longest I have spent away from home, and his support, his calm and genuine kindness meant a lot to me.

From the Finance team to the Clinical Trial Support Group (CTSG), it was a pleasure to work with such professional team at the Mahidol-Oxford Tropical Medicine Research Unit (MORU). I'm grateful to Nick Day and Stuart Blacksell for their encouragement during these 5 years in Asia, as well as to Janjira Thaipadungpanit, my laboratory mentor, who spent hours teaching me how to correctly manage samples despite my obvious lack of skills. I would not have been an Oxford DPhil student without the support of Karen Valentine, previously the Chief Operating Officer at MORU, and I will not forget her critical role in this thesis.

I am grateful for the collaboration with Matthew Rubach on the Tanzanian Study: his kindness and patience for finalising and publishing the report were a great example of resilience.

My deep appreciation goes out to the local team members without whom this thesis would not have been possible: in Myanmar, Kyaw Soe, Myo Maung Maung Swe, Myo Min, James Heaton, Joshua Cohen, Elizabeth Ashley, Frank Smithuis, Ni Ni Tun, and in Thailand; Keng, Bee, Areerat Thaiprakhong, Nattapon Pinthong, Rachel Greer and Tri Wangrangsimakul. Thank you to Andrea, Liz, and Chanaki for their help in revising this thesis.

On a more personal note, I wanted to acknowledge the importance of my family in France, who have always been my refuge during this thesis. Adapting to a new life both professionally and culturally was not easy, and the feeling of family closeness was priceless. A special thanks to Stije, James, Thomas and Emile for their affection, their energy and enthusiasm. Inke, finally, who accompanied me every day in the good and bad moments of my last year writing this thesis.

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LIST OF ABBREVIATIONS

AFI	Acute febrile illness
ALMANACH	Algorithm for the management of Childhood illness
AMR	Antimicrobial resistance
aOR	Adjusted odds ratio
ARI	Acute respiratory tract infection
ASSURED	Affordable, sensitive, specific, user-friendly, rapid, equipment-free, and delivered to those who need it
AUC	Area under the receiver operating characteristic (ROC) curve
AUF	Acute undifferentiated fever
BLB	Bacterial lysis buffer
BSI	Blood stream infection
CDC	Centers for diseases control and prevention
CHWs	Community health workers
CI	Confidence interval
CRF	Case report form
CRP	C-reactive protein
DBS	Dried blood spot
DNA	Deoxyribonucleic acid
DMR	Department of Medical Research
EC	Ethics committees
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme-linked immunosorbent assay
e-POCT	Electronic point-of-care test
FIND	Foundation for Innovative New Diagnostics
FTMEC	Faculty of Tropical Medicine Ethics Committee
G6PD	Glucose 6-phosphate dehydrogenase
GAS	Group A *Streptococcus*
GPs	General practitioners
HIV	Human immunodeficiency virus
HRs	Hazard ratios
ICC	Intra-cluster correlation coefficient
ICD-10	International Classification of Diseases, Tenth Revision, Clinical Modification
ICF	Informed consent form
IFA	Immunofluorescent antibody
IFN-γ	Interferon-γ
IPCO	Internal positive control
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1	Interleukine-1
IL-6	Interleukine-6
IMAI	Integrated management of adolescent and adult illnesses
IMCI	Integrated management of childhood illnesses
ITT	Intention-to-treat

IQR	Inter-quartile ranges
Lao PDR	Lao People's Democratic Republic
LMICs	Low-middle income countries
LRTI	Lower respiratory tract infection
MAM	Medical Action Myanmar
MAT	Microscopic agglutination test
MDR	Multi-drug resistant
MORU	Mahidol-Oxford Tropical Medicine Research Unit
MxA	Myxovirus resistance protein 1
NCBI	National Center for Biotechnology Information
NLCR	Neutrophil lymphocyte count ratio
NP	Nasopharyngeal
OD	Optic densitometries
OPD	Outpatient department
OPS	O-polysaccharide
OUT	Operational taxonomic units
OxTREC	Oxford Tropical Research Ethics Committee
PAMPs	Pathogen-associated molecular patterns
PCR	Polymerase chain reactions
PCU	Primary care units
PHO	Public health office
POC	Point-of-care
POCTs	Point-of care tests
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RBC	Red blood cell
RCTs	Randomised controlled trials
RD	Risk difference
RDTs	Rapid diagnostic tests
RNA	Ribonucleic acid
RNP3	RNase P-3
ROC	Receiver operating characteristics
rRNA	Ribosomal ribonucleic acid
RSV	Respiratory syncytial virus
RT-PCR	Reverse-transcription polymerase chain reaction
RT-qPCR	Quantitative reverse- transcription polymerase chain reaction
SAE	Serious adverse event
SD	Standard deviation
SSA	Sub-Saharan Africa
sTREM-1	Soluble triggering receptor expressed on myeloid cells 1
TAC	Taqman array card
TB	Tuberculosis
TNA	Total nucleic acid
TNF-α	Tumour necrosis factor alpha
TRAIL	Tumour necrosis factor-related apoptosis-inducing ligand
UK	United Kingdom
URTIs	Upper respiratory tract infections
USA	United States of America
WHO	World Health Organization

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Chapter 1

INTRODUCTION

1 INTRODUCTION

1.1 Acute fever & Antibiotic prescription in Southeast Asia

Back in 2010, malaria affected 219 million individuals and caused 660,000 deaths worldwide, according to the World Health Organization (WHO) [1]. With the extensive use of malaria tests at point-of-care (POCT) and access to effective anti-malarial drugs, health workers increasingly face the challenge of febrile patients with a negative malaria test [2-4]. In Southeast Asia, malaria incidence showed the largest decline in the world by nearly 50% between 2010 and 2016, and only 6% of the deaths due to malaria occurred in this region in 2016 [5]. However, fever remains a common cause for seeking healthcare in this region both at the hospital level [6, 7], as well as in the community: over 80% of acutely ill patients attending primary care in Chiang Rai, Northern Thailand, present with clinical symptoms of an infection [8]. The burden of acute fever is likely to increase in Southeast Asia: population density reached 660 million inhabitants living in a tropical climate in 2018, and 155.4 million international visitors are anticipated to travel to this region in 2022, facilitating pathogen spread [9]. Additional risk factors include significant heterogeneity in socio-economic development with large pockets of poverty and limited access to health care; unregulated urbanisation and deforestation, as well as intensive livestock production often in close quarters to human habitation, favouring zoonotic diseases [10, 11]. Last but not least, over 60-year-old individuals are predicted to double between by 2050, reaching 2.1 billion globally [12]. Comorbidities commonly found in older people alter immunity, such as diabetes and cancer, may therefore worsen the burden of febrile illness.

Defining the burden of febrile illness is itself challenging. A 2019 publication in South India highlighted that “Fever in the tropics is a nebulous terminology” and that concepts like “acute febrile illness” (AFI) lack clarity, with an absence of consensus both on what symptoms define AFI, and what duration distinguishes acuteness from chronicity [13]. How elevated a temperature should reach to be classified as fever also varies in the literature, implying that pathogens detected may not be comparable between studies [6, 14].

Febrile illness can be due to a variety of causes, but most commonly to infections with a broad diversity of pathogens including bacteria, viruses, fungi and parasites. Their identification is a challenge in Southeast Asia, as more than 50% of febrile patients do not have any pathogen detected even in well-resourced research studies. Most of these studies are hospital-based, implying particular pathogens and generally higher levels of patient comorbidities and severity; findings might therefore be of limited generalisability to patients attending community-based healthcare settings [6, 7, 15, 16]. Primary care patients are usually non-severe and attend early after symptom onset, implying low pathogen loads, lowering the chance to detect the pathogen causing fever [17-24]. In addition to a poorer understanding of the causes of febrile illness, primary health care is also defined by a shortage of human resources, diagnostics, and evidence-based management guidelines, particularly in low-to middle-income countries (LMICs) [25]. This directly affects the management of febrile patients attending the first lines of care.

Scaling up the means to identify the causal pathogen among patients attending basic levels of care may be unrealistic, but improving fever management in these settings might not require systematic identification of the exact cause of illness in all patients. A careful clinical examination following the WHO guidelines for Integrated Management of Childhood Illnesses (IMCI) or Integrated Management of Adolescent and Adult Illnesses (IMAI) may be sufficient to guide health workers. However, access to these guidelines is limited in remote areas due to shortage of material resources, their guidance for febrile patients without any orienting clinical sign lacks clarity, and health workers are not trained to apply these guidelines [26, 27]. Besides, clinical presentations often overlap between bacterial and viral pathogens, and front line health workers often do not benefit from sufficient clinical training on how to identify patients who need an antibiotic [6, 7, 28-32]. A clinical examination also requires time for a comprehensive medical history and physical examination, while primary care health workers often spend less than five minutes per patient in LMICs [33].

Considering these gaps in guidelines, clinical judgment and microbiological structures, identifying febrile patients who need an antibiotic is predictably difficult in primary health care, resulting in irrational prescribing behaviour at rates that far exceed the estimated needs [25, 34, 35]. Additional drivers include patients' expectation for an antibiotic, as we recently demonstrated in

primary care settings in northern Thailand, as well as health workers' fear of missing a bacterial infection, particularly in hard-to-reach areas where patient follow-up is unrealistic [36, 37].

Antibiotic prescription is therefore high in primary care settings, with 50% of viral upper respiratory tract infections (URTIs) treated with antibiotics in Southeast Asia in 2006 [33]. Estimates from 2010-2015 have confirmed similarly high levels across the region: the proportion of antibiotics prescribed for URTIs ranged between 43% in Thailand and 87% in Myanmar [34]. Between 2015-2016, antibiotic prescription was measured at 46.9% among patients with a fever or a history of fever in northern Thailand, while 13.8% were also prescribed an antibiotic despite the absence of fever, history of fever, or sign of infection [8]. Antibiotic use is associated with the emergence of resistant bacteria [38-40], associated with high mortality: in Thailand, over 19,000 deaths were attributed to multi-drug resistant (MDR) bacteria in 2010 [41]. A systematic review of 41 studies in 7 Southeast Asian countries identified an increase in MDR *Acinetobacter baumannii* compared with other regions (reaching 58.5%), with a 1.7 times greater mortality rate compared with drug-susceptible *Acinetobacter baumannii* [42]. Overall, Southeast Asia is anticipated to bear the greatest mortality burden due to antimicrobial resistance (AMR) worldwide by 2050, although global estimates of the AMR attributable mortality are unreliable [43, 44]. On the other hand, common but potentially lethal tropical bacterial illnesses such as melioidosis, leptospirosis or scrub typhus remain untreated [6, 7, 45, 46]. This indicates a double threat from poor management of febrile patients in low-level care, due to both under- and over prescription of antibiotics.

Although some high-income countries have designed strategies that reduce the AMR burden by restricting antibiotic prescriptions to patients who really need them [47-49], these may need to be adapted given the context of LMICs, by deploying locally relevant and cost-effective interventions [50]. Primary care settings are the largest provider of antibiotic prescriptions both in high-income countries and LMICs [34, 51-53]. Improving fever management by implementing interventions adapted to these regions is therefore needed.

1.2 Point-of-care tests & C-reactive protein

POCTs may represent an attractive prospect in guiding health worker's decision at the time of the consultation, by addressing limitations in clinical judgment and laboratory access [25]. However, several conditions have to be met prior to any routine use: POCTs should positively impact patient outcomes, be validated in the specific environment of the healthcare and population where they are intended for subsequent use, and also be cost-effective [54]. This explains why the transition from design of tests with good analytical performance to their adoption in routine care takes years [55]. In the context of non-malarial febrile illness, POCTs may help health workers identifying febrile patients with a bacterial infection, for whom an antibiotic should be prescribed. At the moment, most POCTs only target a single pathogen and often do not distinguish past-exposure from active infection [16, 56]. For instance, scrub typhus commonly causes AFI in Southeast Asia, due to a bacterium named *Orientia tsutsugamushi* [57]. Currently available POCTs for scrub typhus are antibody-based, with false negative results occurring in patients due to early attendance with slow antibody rise, and false positives due to past exposure and low cut-off titres undermining their ability to diagnose an active infection in endemic regions [58]. A 2018 systematic review and meta-analysis on scrub typhus POCTs highlighted important heterogeneities in diagnostic performance, and urged for the development of an "affordable and accurate POCT" [59]. Multi-pathogen testing based on nucleic acid detection or whole genome sequencing may represent a solution to explore the large diversity of non-malarial pathogens. Evaluations, however, require budget and laboratory capacity that are limited in LMICs [60, 61]. Moreover, interpretation of these data is challenging: a recent paediatric study on the causes of community-acquired serious infections detected bacteria and viruses both in febrile and healthy infants, using a multi-pathogen molecular diagnostic tool in Asia [62]. This raises the question of the actual contribution of the detected organism to the current illness.

Infections caused by bacteria, viruses or other pathogens trigger host-immune biological responses, which can be analysed by measuring various markers such as lactate, white blood cells and their differentiation [63, 64]. Potential advantages of testing for these markers include low cost, short

time for results and availability in settings with a basic capacity. However, none of these markers showed sufficient accuracy in identifying bacterial infections [63-65]. A meta-analysis including 3,320 patients from five studies assessed the neutrophil lymphocyte count ratio (NLCR): its performance for identifying bacteraemic patients was moderate (area under the curve [95% confidence interval], AUC 0.72, [95% CI 0.69-0.74]), in line with a large study on 1,954 patients including 270 with a bacteraemia [65, 66]. All of this evidence originates from hospitals in high-income countries, and suggests limited clinical utility.

Some biomarkers of the immune host-response have been evaluated in broader conditions of populations and settings, although none has demonstrated consistently high diagnostic performance [54, 67]. Procalcitonin for instance, has been well-evaluated with the objective to distinguish between bacterial and viral infections: a large review of the literature identified wide performance variations especially in terms of sensitivity (ranging from 38-97%), and most studies were restricted to patients with a respiratory presentation [67]. A meta-analysis of 30 studies calculated an AUC 0.85 (95% CI 0.72-0.81) with 77% sensitivity and 79% specificity, but only to differentiate between sepsis and systemic inflammatory response from a non-infectious origin, and not between bacterial and viral pathogens [68]. Overall, these reviews and meta-analyses mainly included hospitalised patients from high-income countries, and did not agree on a consensus for any threshold. Another biomarker is the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), which is up-regulated during viral infections and has recently shown promising performances [69, 70]. Here again, evidence is hospital-based in high-income countries without any consensus for a threshold.

C-reactive protein (CRP) is also a host-response biomarker, discovered in 1930 by William Tillett and Thomas Francis from the Rockefeller Institute, as the “fraction C” isolated among patients infected with pneumococcus [71]. The first suggestion of CRP as a biomarker of inflammation was made in the 1940s, based on observations among patients suffering from myocarditis and rheumatic fever, and was suspected to be a marker of atherothrombosis among patients with an acute myocardial infarction a few years later [72, 73]. In the literature, the clinical significance of CRP is particularly well-described in cardiovascular diseases, including diabetes and coronary artery disease risk evaluation, as well as in inflammatory illnesses such as rheumatic arthritis, or inflammatory bowel

disease [74-77]. In brief, CRP is produced by hepatocytes, with the potential to increase 6 hours after a stimulus, and reflects ongoing inflammation without diurnal variation or alteration in patients with comorbidities, including cirrhosis [74, 78]. In the context of febrile illness, bacterial infections activate pathogen-associated molecular patterns (PAMPs) during an initial recognition phase, stimulating inflammatory cytokines especially interleukine-6 (IL-6), which is then stimulating CRP to recruit complement system, stimulate phagocytosis and induce an inflammatory response. Viral infections trigger distinct PAMPs, which induce the production of molecules like type I interferons and interferon-γ (IFN-γ) [70]. This distinction in activation pathways is not absolute, with viral pathogens also stimulating IL-6, explaining why CRP has never been demonstrated to be a perfect discriminatory tool between bacterial and viral pathogens [70].

Despite its early discovery in the 1930s and being one of the most studied host-response biomarkers for predicting bacterial infections, the evidence on CRP from LMICs remains limited [67]. Considering primary care settings, high-income countries remain the main source of evidence, which is limited to patients with a respiratory presentation [79, 80]. At the time of my initial literature review in 2015, the few studies evaluating CRP in febrile patients in LMICs were hospital-based, using small sample sizes, and retrospectively measuring CRP concentrations either in specific age categories and/or in particular clinical presentations [67, 81-85]. These consistently showed high sensitivity and moderate specificity. At the patient level, this implies a potential risk of over treatment in case of a viral infection, but a low risk to miss a bacterial infection.

Because most CRP evaluations on febrile illness originate from hospitals in high-income countries, findings are not applicable to ambulatory patients in LMICs. For instance, hospitalised patients tend to be clinically more severe than outpatients attending primary care, with different inflammation levels [86-88]. These also vary according to various internal and external factors, such as ethnicity, age, gender but also temperature, diet and nutritional status [89-92]. A comparative study showed that healthy Ghanaians living in rural malaria-endemic areas had lower baseline CRP concentrations than healthy Dutch (0.98 *versus* 1.52 mg/L, $p < 0.001$), but higher concentrations during an acute phase response (24.6 *versus* 17.3 mg/L, $p = 0.04$) [93]. Other studies have shown that chronic pathogen exposure may influence baseline CRP concentration [94, 95]. Whether a low-grade

inflammation increases the susceptibility to infectious diseases remains unclear, but the continuous interaction between an individual and their environment is acknowledged to impact the individual immune system. Another component affecting CRP concentration during the acute phase is the pathogen itself, by triggering a specific adaptive immune response through distinct inflammatory reactions [96]. For these reasons, interpretation of inflammatory biomarker responses should be restricted to the particularities of the population, setting and pathogen spectrum in which they are evaluated.

CRP-testing has also been assessed with the objective of improving prescribing practices regardless of the aetiology and based on clinical recovery, particularly in primary care. A systematic review including six trials (3,284 patients but only 139 children) measured a significant antibiotic reduction after testing for CRP during acute respiratory infections without affecting patient outcome, although a precise estimate of such reduction was not possible [79]. Another analysis demonstrated the cost-effectiveness of CRP POCT among adults with acute cough and lower respiratory tract infection [97]. These findings originate from high-income countries and mostly focus on adults. More recently, three trials of CRP-testing have been published in primary care in LMICs: the first one was carried out in 2016 among 2,036 children and adults presenting with a non-severe acute respiratory tract infection in Vietnam [98]. Antibiotic prescription was reduced by 14 percentage points compared with standard-of-care, without altering patients' clinical outcomes, using a 10 mg/L threshold for children and 20 mg/L for adults. The second trial included over 3,000 children from 2-59 months of age in Tanzania, evaluating the impact of an electronic algorithm with a package of point-of-care interventions (e-POCT), including pulse oximetry, glucometer, malaria rapid test, haemoglobin, procalcitonin and CRP [99]. When compared with a validated electronic algorithm derived from the IMCI named ALMANACH (algorithm for the management of childhood illness), the e-POCT significantly reduced antibiotic prescription and improved clinical outcomes (risk ratio 0.57, 95% CI 0.38-0.85). The third study extended CRP POCT evaluation to all febrile children and adults [100]: it is central to this Thesis and will be described in the following chapters.

Beyond scientific validation, WHO identified several criteria prior to deploying a POCT, known as ASSURED (affordable, sensitive, specific, user-friendly, rapid, equipment-free, and

delivered to those who need it) [101]. Commercially available CRP POCTs exist, with some costing under 1 US dollar (https://www.finddx.org/wp-content/uploads/2018/03/CRP_List_2018_02_26.pdf) and being cost-effective even in LMICs, while showing similar accuracy when compared to CRP gold standard methods [102, 103]. By contrast, testing for procalcitonin costs approximately 15 US dollars, preventing any deployment in LMICs [67, 104]. Another inflammatory biomarker named Myxovirus resistance protein 1 (MxA) is elevated during viral infections and is potentially available at point-of-care, with evidence showing good diagnostic performance when combined with CRP, but its evaluation relied on small sample sizes, restricted to hospitals in high-income countries [105, 106].

1.3 Thesis narrative & Overview

1.3.1 First steps in tropical medicine

I joined the Mahidol-Oxford Tropical Medicine Research Unit (MORU), Bangkok, Thailand in April 2015, after 10 years treating febrile patients in Paris and a field experience in Liberia as a dead body management (DBM) officer with WHO, during the Ebola virus epidemic.

My main role at MORU was coordinating the CRP Study, a randomised controlled trial of CRP POCT among non-malarial febrile children and adults attending primary health care in Thailand and Myanmar [100]. The primary objective was to evaluate whether CRP POCT could improve antibiotic prescription. "Improving" antibiotic prescription does not only mean reducing prescriptions, but also increasing the antibiotic coverage of patients with a bacterial infection.

Prior to the CRP Study, I was involved in an ongoing project evaluating CRP and procalcitonin performance in differentiating bacterial from viral pathogens [107]. This study was the first to include children and adults from LMICs, including Cambodia, Lao People's Democratic Republic (PDR) and Thailand, with 1,372 microbiologically-confirmed specimens from both inpatients and outpatients. CRP diagnostic accuracy for detecting bacterial pathogens was significantly higher than procalcitonin, with an area under the receiver operating curve (AUROC) calculated at 0.83, 95% confidence interval (0.81-0.86) *versus* 0.74 (0.71-0.77), p-value <0.001. CRP performance was even greater when considering bacterial blood stream infections (BSI) and not bacterial zoonosis, with an AUROC 0.89 (0.80-0.97), as compared with 0.78 (0.65-0.90) for procalcitonin. The study was also evaluating different thresholds: for CRP, the sensitivity and specificity were 95% and 50% respectively for a threshold of 10 mg/L, and 86% and 67% for a threshold of 20 mg/L. By contrast, procalcitonin showed a sensitivity and specificity of 90% and 39% respectively using a 0.1 ng/mL threshold, and 60% and 76% respectively using a 0.5 ng/mL threshold (Figure 1.1).

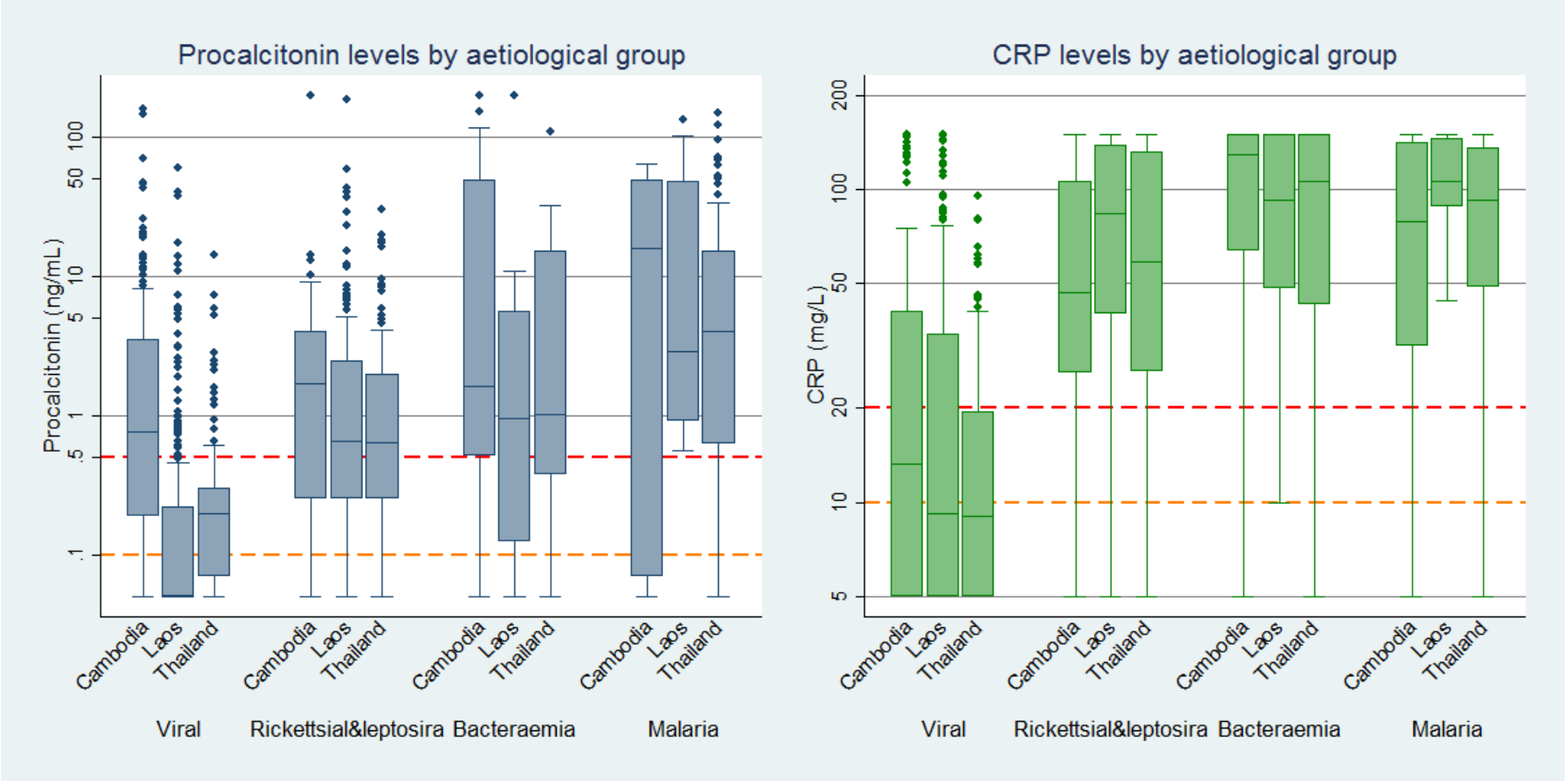


Figure 1-1 Procalcitonin and C-reactive protein levels in the four aetiological groups in Cambodia, Lao PDR and Thailand

Several comments should be made regarding this study: microbiological investigations and laboratory procedures varied across countries; specimens from outpatients originated only from children over 5 years of age; in Lao PDR, health centres recruiting outpatients were hospitals and not primary care settings; and both Lao PDR and Thai-Myanmar border outpatients with a clear clinical presentation such as diarrhoea or acute respiratory infection were excluded.

Following this first step, I was involved in a modelling study of the potential impact of CRP POCT on antibiotic prescription in the tropics [102]. Using data on causes of fever in rural Laos, we compared CRP POCT with the most validated pathogen-specific rapid diagnostic tests (RDTs) including the dengue and scrub typhus rapid tests. Despite an assumption that these pathogen-specific RDTs had a high accuracy, sets at 95% sensitivity and specificity, testing for CRP would have outperformed these RDTs by reducing unnecessary antibiotics in viral infections, as well as lowering the proportion of untreated bacterial infections (Figure 1.2).

Using CRP POCT, 80% of patients would have been correctly treated compared with 52% in current practice, 46% using a dengue virus RDT and 59% using a scrub typhus RDT. The model was repeatedly run in 500 scenarios with varying prevalence of dengue, scrub typhus and other common tropical infections, but in almost all scenarios, CRP-testing was the superior approach. We also carried out a cost-effectiveness analysis, using the cost of RDTs and a course of antibiotics, as well as the mortality and excess illness duration in the absence of an effective antibiotic. Only the scrub typhus and CRP tests were estimated to be cost-effective.

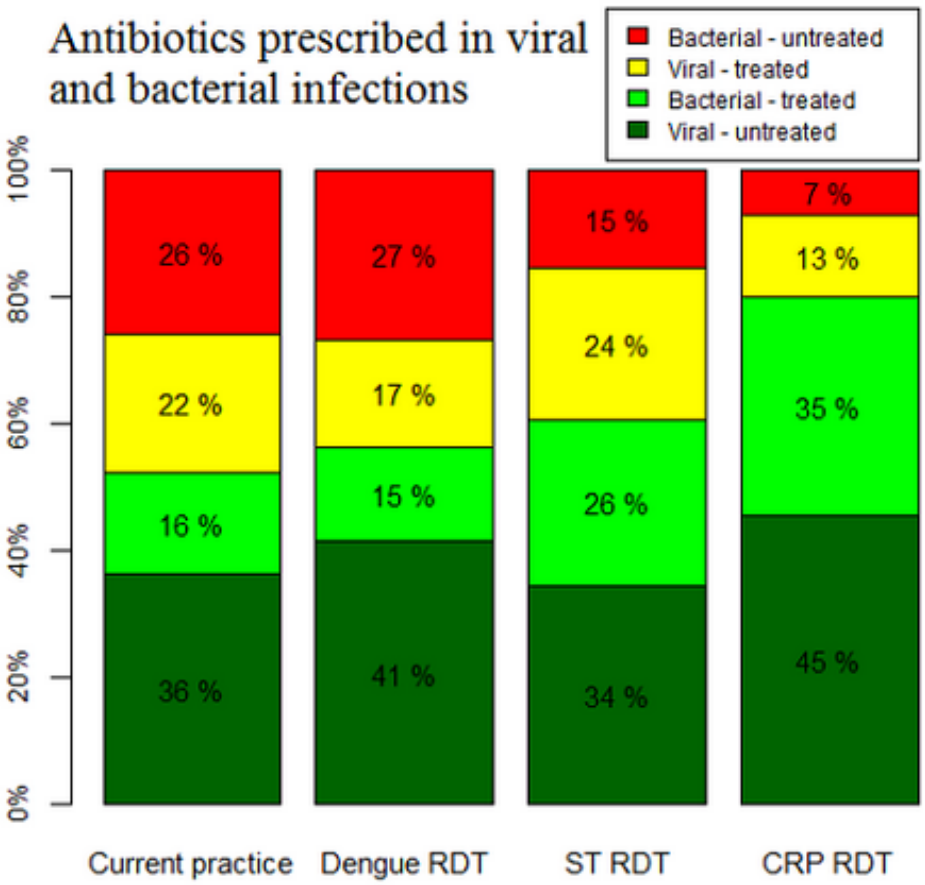


Figure 1-2 Antibiotic targeting using the Laos data, with different tests and their impact on the proportion of correctly treated patients

ST–scrub typhus; CRP–C reactive protein; RDT–rapid diagnostic test.
The bottom dark green segment of the bars shows the percentages of patients with viral infections not prescribed an antibiotic; the light green segment shows the percentages of patients with bacterial infections correctly prescribed an antibiotic; the yellow segment shows the percentages of patients with viral infections prescribed an antibiotic; and the red segment shows the percentages of patients with untreated bacterial infections.

In parallel to this project, I participated in an assessment of the accuracy of commercially available rapid tests for CRP, using specimens from the 686 febrile Laotian patients detailed above [102, 108]. Three CRP POCTs were evaluated on specimens taken at patient presentation, followed by a second measurement with the reference test (Nycocard Reader II, Axis Shield, Norway). The sensitivities ranged from 87-98% and specificities from 91-98%, when interpreted in a binary manner using a threshold of 10 mg/L. Two out of the three RDTs were semi-quantitative and both showed high agreement with the reference test result, with a weighted kappa ranging from 0.7-0.8. Results are presented on Figure 1.3. Although CRP semi-quantitative tests offer more information about the patient's inflammation level, their interpretation in low levels of care without a trained dedicated reader may be challenging [109]. Interestingly, antibiotic prescription was 58.8% while two out of the three sites showed CRP levels below 10 mg/, indicating that the potential for antibiotic reduction is high.

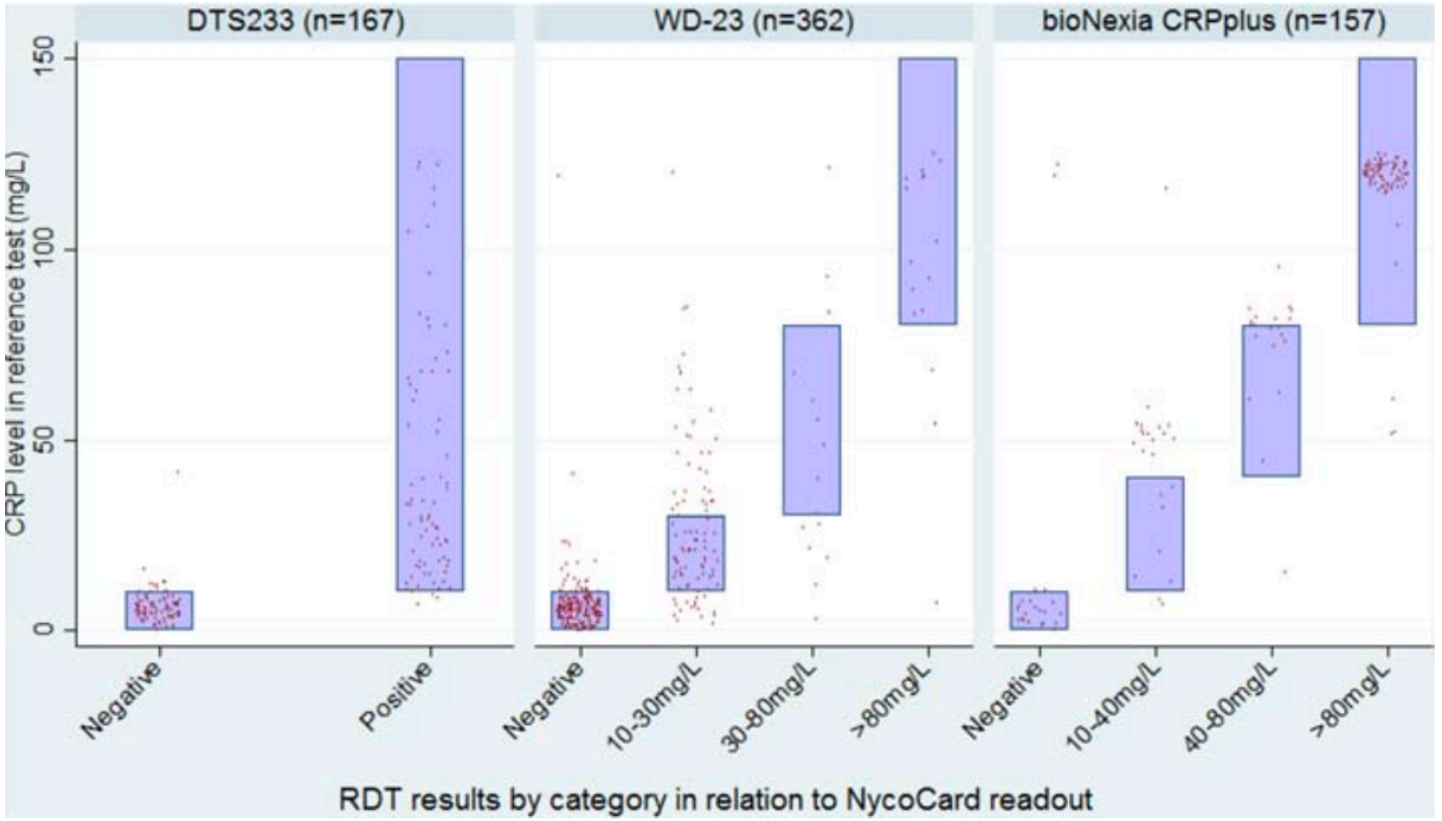


Figure 1-3 Results rapid tests in relation to quantitative reference test (Nycocard Reader II, Axis-Shield, Norway)

Finally, I was fortunate to be part of another investigation on causes of acute undifferentiated fever (AUF) and CRP performance in Prachanukroh hospital, Chiang Rai, Northern Thailand [110]. Regarding this observational study, my role was limited to data analysis, and not study design or sample management. Hospitalised patients over 15 years of age with AUF were recruited between 2006-2008, with the objective to inform about the key pathogens in the region, as data from northern Thailand are limited [107]. Scrub typhus, dengue virus and leptospirosis were the leading AUF illnesses. Another aspect of the study aimed to evaluate whether CRP and procalcitonin could discriminate between bacterial and viral pathogens: CRP showed a sensitivity between 86-92% and specificity between 73-86% for a threshold of 20 and 40 mg/L, respectively. The AUC was measured at 0.91, with 95% confidence interval (0.85-0.96). Performance of procalcitonin was of lesser accuracy. Furthermore, the potential impact of these two biomarkers on antibiotic prescription was estimated compared with the current practices: with 75% of patients with a viral aetiology receiving antibiotics, the potential utility for CRP safely reducing antibiotic prescription appeared high.

1.3.2 In Southeast Asia: the CRP Study

Alongside my Thesis, the term "Southeast Asia" will refer to ten countries including Brunei, Cambodia, East Timor, Indonesia, Lao People's Democratic Republic (PDR), Malaysia, Myanmar, Philippines, Singapore, Thailand and Vietnam, according to the Association of Southeast Asian Countries (ASEAN) [111]. The term "primary care" will refer to the first level of contact with a national health system, providing health care as close as possible where people live and work. The provision of such care should be universally accessible to the community, and the community should participate in the identification and sustainable management of health issues [112].

My involvement in these initial studies enhanced my understanding of CRP for its potential use and limitations. For instance, none of these studies evaluated the performance of CRP in discriminating bacteria from viruses among outpatients of all ages in primary care settings, and regardless of their clinical presentation. The question, namely whether CRP POCT could improve antibiotic prescription among all febrile patients in the tropics, was unanswered at that time. Later in 2016, a randomised controlled trial in Vietnam evaluated the impact of CRP-testing in primary care children and adults

presenting with a non-severe acute respiratory tract infection (ARI) [98]. Participants were prospectively randomised either to the CRP POCT or standard-of-care group, and followed-up over 14 days. The objective was to evaluate the impact of CRP POCT on antibiotic prescription without altering patient clinical outcome, rather than assessing the diagnostic performance for discriminating between bacterial and viral pathogens. In this study, antibiotic prescription was reduced from 78% in current practice to 64% using CRP POCT with a 10 mg/L threshold in children and 20 mg/L in adults, without altering symptom duration or the occurrence of serious adverse events. This 14 percentage point reduction in antibiotic prescription was of a similar magnitude to comparable studies in high-income countries [113-116]. Substantial heterogeneity in the impact of CRP was identified between the sites, with unequal adherence to CRP results. This highlights the importance of the local context in such intervention, as well as the need to combine CRP-testing with additional measures. For instance, education, supervision and communication skills training have been identified as effective in complementing the impact of CRP on prescribing behaviours in high-income countries [117-119].

With these findings and limitations in mind, I planned my Thesis with the objective to extend the evaluation of CRP both as a biomarker for differentiating bacterial from viral pathogens, but also as a clinical tool guiding health worker's antibiotic prescription regardless of the aetiology and based on clinical outcome, given the context of acutely febrile children and adults attending primary care in LMICs. I therefore participated in the design, implementation and outcome analysis of the CRP Study, a multi-site randomised controlled trial in Thailand and Myanmar. In total, we recruited 2,392 children and adults attending primary care, excluding under 1-year-old patients, due to the limited evidence on CRP in this age category. Any patient with either a documented fever (defined as a tympanic temperature >37.5˚C) or a history of recent fever (within the last 14 days) was included.

The CRP Study aimed to inform about the key pathogens causing fever in low level of care in Southeast Asia, which few previous studies addressed: Mueller et al. (2014) included three primary care sites in one country (Cambodia) and excluded children under 7 and adults over 49 years of age [21]. Pathogens reported by Lubell et al. (2015) originated from hospitalised patients in Cambodia, while those from Lao PDR were from two district hospitals only and not from any primary care centre; paediatric outpatients from Lao PDR and the Thai-Myanmar border were all over 5 years of age; and

patients with a clear clinical sign apart from fever were excluded [107]. We therefore extended CRP evaluation to all febrile patients across 10 primary care sites in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar. Blood specimens were taken to determine the causes of acute fever, as well as nasopharyngeal swabs, because a substantial proportion of primary care patients present with an ARI in Southeast Asia [8].

The second objective of the CRP Study was to assess CRP accuracy in distinguishing bacterial from viral pathogens. In Southeast Asia, only Lubell et al. (2015) evaluated CRP accuracy using different thresholds among outpatients [107]. However, this evaluation included outpatients with an undifferentiated fever, and only one country out of three enrolled patients from primary care settings. In our study, we included all febrile children and adults attending primary care regardless of their clinical presentation, and we also provided an optimal threshold for each clinical syndrome including neurological, respiratory and digestive presentation.

The third and primary objective of the CRP Study was to prospectively evaluate the impact of CRP POCT on antibiotic prescription. The previous and only prospective evaluation was implemented in Vietnam, limited to ARIs, and evaluated one threshold per age category [98]. We extended this evaluation to all febrile patients regardless of clinical presentation, and assessed two different thresholds both in children and adults (i.e. 20 mg/L and 40 mg/L). The primary endpoint was the difference in antibiotic prescribing between the two intervention arms and controls. Detailed methods, results and limitations of the CRP Study will be described in the following chapters.

1.3.3 In sub-Saharan Africa: the Tanzanian Study

As mentioned previously, the management of febrile illness has been changing over the last decades in Asia, mainly due to the reduction of the malaria burden [2, 4, 56]. A similar trend is observed in sub-Saharan Africa, and although most malaria cases and deaths occur in this region, according to WHO, the proportion of *Plasmodium falciparum* has been reduced among acutely febrile patients [120]. In Tanzania, over 60% of febrile children and adults attending two regional hospitals in 2013 were clinically diagnosed with malaria but less than 2% of cases were microscopically-confirmed to be malaria, while a paediatric study investigated 1,005 febrile children attending two outpatient clinics in

2014, and only identified *P. falciparum* in 10.5% of cases [23, 121]. Whether or not CRP testing could be useful in the management of febrile illness in sub-Saharan settings has not been well established.

A few studies focused on HIV-infected patients, with the objective to rule-out tuberculosis as an opportunistic infection [122-124]. A few others assessed whether CRP could identify an acute bacterial infection, but were mainly paediatric, hospital-based and restricted to patients with a particular clinical presentation [125-130]. Moreover, bacterial pathogens were only detected using blood culture and not serological or molecular methods, excluding zoonotic organisms from CRP evaluations [131-133]. In general, CRP concentrations have been reported to be significantly elevated among hospitalised children with a bacterial infection, however, no threshold nor diagnostic performance was carried out. Altogether, this suggests a lack of clinical usefulness and warrants further evidence prior to recommending CRP or any host-response biomarker testing in the management of non-malarial febrile illness in sub-Saharan Africa. This also indicates scant information in low levels of care. Nevertheless, at the time of my literature review in 2015, no study prospectively or retrospectively evaluated the impact of CRP POCT on antibiotic prescription in sub-Saharan Africa, regardless of the level of care.

Considering this context, I have had the chance to analyse data from an observational study in Moshi, Northern Tanzania [134]. This study originally enrolled 1,754 febrile children and adults, both inpatients and outpatients regardless of their clinical presentation between 2011 and 2014. Of these, 804 had sufficient blood volume for CRP testing and microbiological investigations, including blood culture and bacterial serology for *Brucella* spp., *Leptospira* spp., *Coxiella burnetii* and rickettsial pathogens. Out of 804 patients, 107 (13%) had a laboratory-confirmed diagnosis. My role in this study consisted of data analysis and not study design, implementation, nor laboratory analysis. This study had several components, including the investigation of the key bacterial pathogens causing acute fever, including bacterial blood stream infections (BSI) and bacterial zoonoses. The second component of this study measured CRP concentrations in regards to the aetiology, as well as CRP sensitivity in identifying these bacterial pathogens for different thresholds. The third component consisted of estimating the potential impact of CRP POCT compared with current prescribing practices, for different types of patients (inpatients and outpatients) and pathogens (bacterial BSIs and zoonoses). Detailed methods, results and limitations of these three components will be described in the following chapters.

1.4 Research questions & Objectives

Following this Introduction section, this Thesis will aim to address specific research questions, such as follows:

- What are the key non-malarial pathogens causing acute fever among children and adults attending the primary levels of care in Southeast Asia?
- Within this particular context, what is the diagnostic accuracy of CRP for differentiating bacterial from viral pathogens?
- Can CRP POCT better target antibiotic prescription among all febrile patients attending the primary levels of care, regardless of aetiology and clinical presentation?

The objectives of this Thesis are aiming to answer those research questions:

- To identify the key non-malarial pathogens causing acute fever among children and adults attending the primary levels of care in Southeast Asia.
- To evaluate the diagnostic accuracy of CRP in differentiating bacterial from viral pathogens, in the context of febrile and non-severe patients attending the primary levels of care both in Southeast Asia and sub-Saharan Africa.
- To measure the impact of CRP POCT on antibiotic prescription, given the context of acutely febrile patients attending the primary levels of care.

Chapter 2

LITERATURE REVIEW

2 LITERATURE REVIEW

In this chapter, three literature reviews will be presented, including on causes of fever in Southeast Asia, performance of C-reactive protein (CRP) to discriminate between bacterial and viral pathogens in the tropics, and impact of CRP-testing at point-of-care (POCT) on antibiotic prescription in primary care. These reviews were key in my thesis, especially for identifying the main limitations of acutely febrile patient management in the context of resource-poor settings.

2.1 Causes of fever in Southeast Asia

2.1.1 Research in context

Global investment for identifying, treating and preventing malaria cases has increased recently, reaching US$ 3.1 billion in 2017 [135]. In parallel, malaria incidence has halved between 2010 and 2016 in Southeast Asia, where the associated mortality does not exceed 6% of global malaria deaths [5]. The identification of malaria cases mainly relies on validated, accurate, affordable, and easy to use diagnostic tools such as microscopy or rapid diagnostic tests (RDTs), directly detecting the presence of the parasite or its antigens. Diagnosing malaria is therefore feasible at point-of-care, even in hard-to-reach areas and low-level care [136, 137].

By comparison, only a few non-malarial pathogens can be detected by point-of-care tests, and several limitations undermine their utility in disease surveillance and control: most bacterial POCTs cannot distinguish past-exposure from active infection because they are antibody-based [56, 58, 59]. Scrub typhus is a common bacterial infection in Southeast Asia caused by *Orientia tsutsugamushi* [57], but a 2018 review and meta-analysis on corresponding POCTs showed inconsistent performances [59]. With a sensitivity ranging between 20-99% using total antibody measurement, the risk of missing a potentially lethal infection is substantial, and is worsened considering false negative patients attending early after symptom onset with initially negative immunoglobin M (IgM). On the other hand, specificity varies between 67-100% with substantial potential numbers of false positive results, increasing

antibiotic misuse based on an immunological "scar". Furthermore, reference techniques used to evaluate scrub typhus rapid tests often rely on indirect immunofluorescence assay (IFA) with low cut-offs (≥1:400 instead of ≥1:3,200), altering the interpretation of RDT results, especially in endemic areas like Southeast Asia. Similarly, a study evaluated four antigen-based tests for the direct identification of *Salmonella enterica* in blood [138]: none of them reliably detected this bacterium even in high concentrations (10^8 CFU/mL), nor did they detect *Salmonella* Paratyphi A, which causes more infections than *Salmonella* Typhi in Asia [139]. Moreover, these tests require cultured blood broth, which is not realistic in the first lines of care where laboratory infrastructures are limited. Other bacterial RDTs have been evaluated, screening for group A *Streptococcus* (GAS), *S. pneumoniae* or *Legionella* spp, but showed inconsistent performance [140-143]. Among viral POCTs, dengue virus is detected using a combination of antibody and antigen (i.e. immunoglobin M and non-structural protein 1), but diagnostic performances vary widely [144], and a recent systematic review on cost-effectiveness has not shown any benefit [145]. Other antigen-based viral POCTs showed inconsistent sensitivities, including for influenza virus A, respiratory syncytial virus (RSV) or Epstein-Barr virus (EBV [146-149].

2.1.2 Geographical heterogeneity

While these POCTs are specific to a single organism, the spectrum of non-malarial causes of fever is broad, therefore achieving high levels of pathogen identification requires a combination of several diagnostic platforms, including blood culture, molecular and serological methods. Considering LMICs in general and Southeast Asia in particular, access to such platforms is challenging: in Myanmar, laboratory facilities are uncommon with poor pathogen surveillance and control, illustrated by outbreaks of common pathogens including influenza virus (A/H1N1 in 2017), Japanese encephalitis virus (2014 and 2016) and dengue virus (2001, 2013, 2015 and 2019) [150]. This gap in diagnostic capacities may be linked to severe clinical outcomes: while investigations on infectious diseases are limited in Myanmar, tuberculosis and lower respiratory infections were identified as the third and fifth leading causes of death in 2015 [151]. Considering Southeast Asia, a systematic review covering 25 years between 1986-2011 identified 146 studies informing on non-malarial pathogens [152]. This

review highlighted a substantial heterogeneity in information distribution throughout the region, with 80% of studies from Thailand and Vietnam. This heterogeneity also included variations in diagnostic techniques used between these studies, while some of them relied on substandard tests and/or thresholds preventing from attributing causality. A more recent review of papers published between 2012-2017 identified 21 studies investigating causes of fever in Southeast Asia [16]. These were restricted to large urban centres, while epidemiological evidence on more peripheral areas remains scarce. Moreover, none of the reviewed studies used the same inclusion criteria nor diagnostic methods, limiting pathogen burden estimates in this region.

Given these recent reviews, my aim in reviewing the literature on causes of fever in Southeast Asia was limited to providing an updated snapshot including pivotal studies published from 2015 to May 2019. I therefore did not follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The search strategy is detailed in Appendix III. Out of 198 studies screened, more than half were rejected because a single pathogen was investigated (107/198, 54%), in consistence with previous literature reviews as it does not provide a global overview of pathogen spectrum. An additional 29 studies (15%) were excluded because microbiological investigations were either not done or not reported. In my analysis, a total of sixteen studies were selected: Thailand was included in seven of them (44%) [15, 41, 110, 153-156]; followed by Vietnam (four studies, 25%) [15, 157-159]; Indonesia was included in three studies (19%) [15, 160, 161]; Lao PDR in two (13%) [6, 14]; Malaysia and Singapore in one study (6%) [162, 163]. It is striking that no study has investigated causes of fever in Myanmar, Cambodia, Philippines, nor Timor-Leste over the past five years. In Vietnam, causes of fever studies were exclusively carried out in major urban centres (Hanoi and Ho Chi Minh City), while in Indonesia, only Java was included. In Thailand, the majority of investigations (5/7, 71%) were carried out in the northernmost part of the country.

Besides, most studies were implemented in a single site (12/16, 75%), where all patients were hospitalised in urban centres [6, 14, 110, 153, 155-160, 162, 163]. Studies investigating rural areas were few (3/16, 19%) [6, 41, 154], while the Greater Mekong Sub-region is one of the least urbanised regions in the world with a total of around 326 million inhabitants (https://www.greatermekong.org/greater-mekong-subregion-rural-no-more). All of the 16 studies on causes of fever included hospitals, and two

studies enrolled outpatients [154, 161]. A single study recruited patients from both primary health centres and hospitals [161]. It is likely that pathogens causing mild presentations among outpatients attending primary level of care differ from those detected among patients seeking care in a hospital, indicating a lack of epidemiological information at that level of care in Southeast Asia [6, 7, 15, 16]. Furthermore, studies were mostly centralised in urban tertiary-care hospitals where laboratory capacities are high (11 out 16 studies). Pathogens identified for this particular population may therefore not be those causing fever among non-severe outpatients in rural areas [17-19].

2.1.3 Clinical heterogeneity

None of the 16 reviewed studies between 2015 and 2019 had the same patient inclusion criteria, undermining potential comparisons and limiting the epidemiological comprehension of pathogen distribution across Southeast Asia. For instance, one study recruited patients over 21 years of age with a particular presentation (haemodialysis and vascular access-associated blood stream infection), while another only recruited patients over 15 years of age with a specific comorbidity (systemic lupus erythematosus) in an intensive care unit [156, 163]. Only three studies (19%) prospectively enrolled febrile patients regardless of their clinical presentation or comorbidity, allowing the generalisation of pathogen findings to all febrile patients [15, 153, 159].

Additionally, the definition of fever was inconsistent across studies, further limiting comparability of findings: the method of temperature measurement was sometimes not described (axillary or tympanic); or differed between studies with one choosing an axillary temperature strictly over 37.5˚C, whereas another opted for a tympanic temperature equal or higher than 37.5˚C [6, 14]. One study excluded patients with an "obvious cause of fever" without a clear definition [6]. The term "acute undifferentiated fever" (AUF) was used as an inclusion criteria in two other studies [110, 154] while this presentation lacks a consensus definition: "acute" undifferentiated fever is defined as either a fever resolving within three weeks [164], or less than two weeks [165-167]. The term "undifferentiated" often refers to the absence of organ-specific clinical symptoms, and this raises the question of how to define the absence of any specific symptom, as patients rarely present with fever as a single symptom, and any additional symptom often relates to at least one particular organ. One study

recruited patients with an "acute febrile illness" (AFI) although the symptom duration defining "acute" and the symptoms to be included in "febrile illness" were not described [161]. Similarly to AUF, AFI duration are either fevers of less than two weeks, or less than three weeks without any clear cut-off, and present a similar challenge of defining what an organ-non-specific symptom is [13].

2.1.4 Microbiological heterogeneity

In addition to discrepancies in demographic and clinical inclusion criteria, microbiological strategy varied across studies. Only four studies combined blood culture with molecular and serological assays for investigating causes of fever in Southeast Asia [14, 15, 110, 161], while ten used blood culture as a single method, implying that vector-borne zoonoses such as scrub typhus or dengue virus were not investigated [41, 153, 155-160, 162, 163]. If interpretation of molecular assay results tends to be standardised, based on the direct detection of the pathogen nucleic acid, serological methods suffer from the risk of cross-reaction between pathogens: for instance, confirmed dengue patients may present positive anti-Japanese encephalitis virus (anti-JEV) IgM [168]. Furthermore, distinguishing between past-exposure and active infection is challenging in endemic areas: in Southeast Asia, a cut-off ≥1:3,200 in an admission sample or a four-fold rise to ≥1:3,200 in a convalescent sample is now recommended for ascertaining an acute scrub typhus infection using IFA [169]. This high cut-off allows 100% specificity but sensitivity is not predicted to exceed 81.6%, raising the concern of missing this potentially lethal infection. Of the five studies using IFA for diagnosing scrub typhus, two used a low cut-off (IgG titre ≥128 and/or IgM titre ≥64 in either acute or convalescent sera) [6, 154].

In terms of findings, studies combining blood culture, molecular and serological methods altogether identified a pathogen in 47% of patients on average, varying between 25-60%, bearing in mind that such proportion of positives corresponded to investigations carried out in a majority of hospitalised patients. Studies using blood culture as a single technique were positive in around 9% (1.2-36.1%). Two studies used molecular and/or serological methods without blood culture, and detected a pathogen in approximately 28% of cases (4.4-52%). Rural and peri-urban studies presented a great diversity of pathogens while investigations in strictly urban centres did not include any vector-borne zoonotic pathogens such as *Orientia tsutsugamushi* or dengue virus nor direct zoonotic pathogens such

as *Leptospira* or *Burkholderia pseudomallei* [155-160, 162, 163]. These gaps in screening undermine empiric treatment guidelines in tertiary care settings in strictly urban settings, as many tropical zoonoses are now endemic in all environments, such as scrub typhus or leptospirosis [57, 170]. Pathogen distribution by type of environment (rural or peri-urban *versus* strictly urban) is illustrated in Figure 2.1.

Rickettsiosis were the most frequent tropical zoonosis reported in 7 of 16 studies, and the proportion of *O. tsutsugamushi* was the highest among these with approximately 7% of positives varying between 1.5-25.5%. This finding is consistent with the two previous literature reviews in Southeast Asia. Paradoxically, public awareness of the high prevalence of this pathogen remains poor: a systematic review on antibiotic self-medication found that amoxicillin was the most frequent antibiotic used in the region, while it is not active against this bacterium [171]. Dengue virus was commonly detected in 5 out 16 studies, being detected in 25% of cases and varying between 8-60%. *Leptospira* was detected in 4 out 16 studies with around 4% of positives, ranging from 0.4-7. Zoonotic pathogens were systematically detected whenever they were tested for, highlighting the importance of broadening and ideally standardising the range of assays used.

Enterobacteriaceae were the most widely reported bacterial family in 14 out 16 studies and represented 11% of cases on average, ranging from 0.5-44.7%. Among these, *Escherichia coli* was the most reported bacterium (11 out 16 studies), detected in approximately 8% of cases, ranging from 0.5-20.6%, while *Klebsiella* (either spp. or *pneumoniae*) and *Salmonella enterica* (either serovar Typhi or non-Typhi) were reported in 7 of 16 studies, at around 7% and 4%, respectively. *Staphylococcus aureus* was one of the most reported Gram-positive bacteria (11 out 16 studies), with 12% of positive cases ranging from 0.2-57.6%. *Burkholderia pseudomallei* was only reported in 2 studies while most studies used blood culture (14 out 16), with 2% of positive cases. Blood culture is the main diagnostic technique to identify this bacterium, but lack of awareness and staff training are acknowledged factors undermining its detection [172]. The scarcity of studies reporting *B. pseudomallei* is alarming considering how prevalent this bacterium is in Southeast Asia, with 40% of the 165,000 cases estimated worldwide occurring in this region every year [45].

Panel A: rural or peri-urban settings

Panel B: strictly urban settings

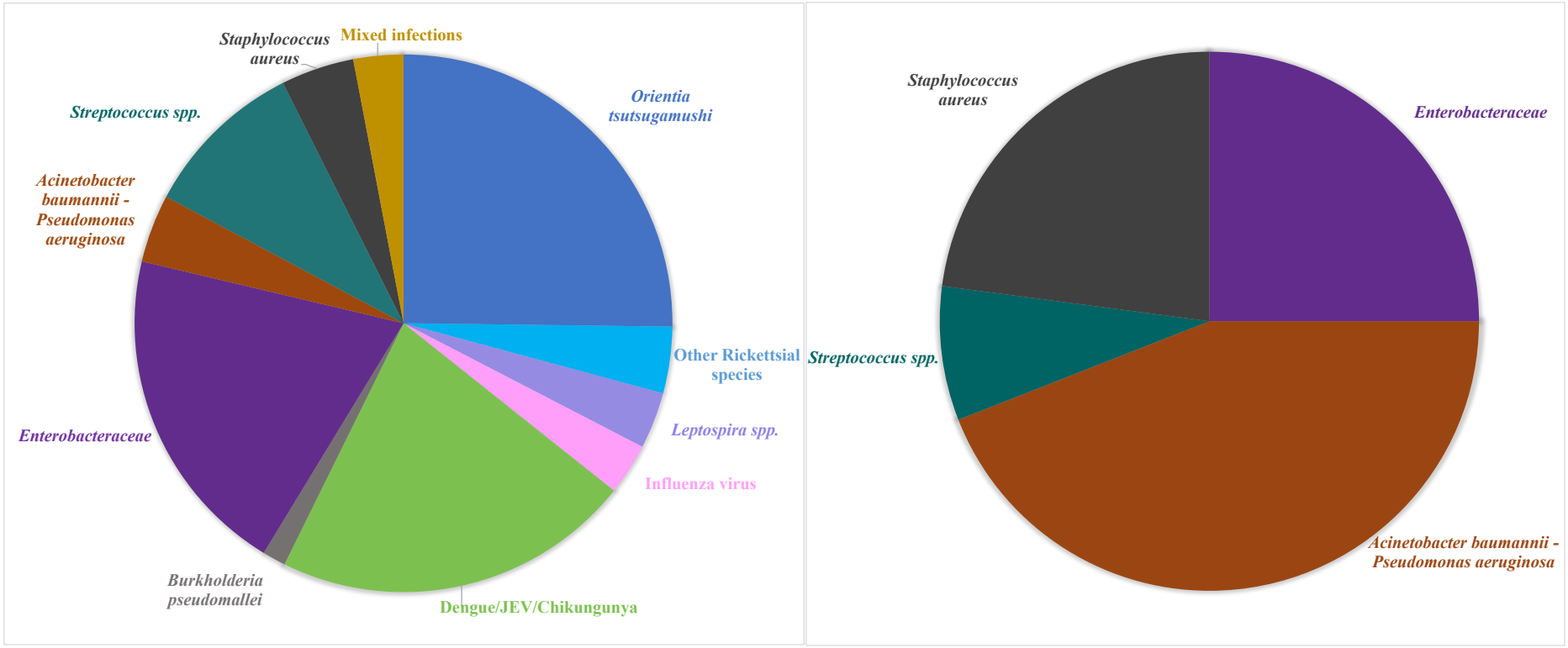


Figure 2-1 Pathogen distribution according to the type of environment (rural or peri-urban *versus* urban) in Southeast Asia

Finally, three studies reported mixed infections, consisting of either mixed vector-borne zoonoses or vector-borne zoonoses and blood stream infections [6, 14, 161]. These infections were very few, with some including organisms detected in respiratory samples considered causal, such as influenza virus, while pathogenicity cannot be ascertained in all cases [173]. Further, some zoonotic pathogens like JEV were confirmed while being diagnosed on IgM enzyme-linked immunosorbent assay (ELISA), although several cross-reactions and diagnostic accuracy limitations are described in the literature [174].

2.1.5 Summary on the causes of fever in Southeast Asia

This 2015-2019 literature review highlighted the importance of vector-borne zoonotic pathogens, especially dengue virus and *O. tsutsugamushi*, as well as direct zoonotic pathogens like *Leptospira* spp. as main contributors of acute fever in Southeast Asia. Studies using blood culture frequently detected *E. coli*, but these were mainly carried out among severe and hospitalised patients.

While the review findings were consistent with its predecessors', additional insights were provided: most causes of fever studies in Southeast Asia are indeed restricted to urban centres while rural regions with hundreds of millions of inhabitants -mainly from the greater Mekong sub-region- are excluded. Primary health centres are often not included in aetiological investigations, whereas this level of care reaches the largest proportion of the general population. Key pathogens causing fever among patients attending primary care therefore remain unknown in Southeast Asia.

Evidence on causes of fever was poorly distributed : while Thailand and Vietnam have been the main providers informing the pathogen spectrum over the last 30 years in Southeast Asia, high-risk regions with dense and mobile populations remain poorly covered, such as the south of Thailand or Myanmar. Pathogen epidemiology has been demonstrated to vary following moderate geographical variations, so it is likely that empiric treatment guidelines for the entire region are not adequate [175]. Regions with gaps in aetiological investigations showed a high burden of infectious diseases, suggesting the need to support diagnostic platforms in the most deprived and resource-poor settings [151].

Inclusion criteria varied across studies with a limited number of studies recruiting patients regardless of clinical presentation, making it challenging to capture the complete spectrum of pathogens among all febrile patients.

Finally, this review underlined the importance of combining molecular and serological methods with blood culture: vector-borne zoonoses are a key driver of acute fever in Southeast Asia, explained by increased agriculture and livestock, as well as by more frequent contacts between humans and wildlife, increasing the propagation of pathogens [176]. A 2017 review on scrub typhus estimated that one billion people were at risk of infection, mainly in Southeast Asia [57]. Most tropical zoonoses such as scrub typhus or dengue virus are detected using serological and molecular methods: in this review, over 60% (10/16) of contemporary studies still relied only on blood culture which does not investigate vector-borne zoonotic pathogens [41, 153, 155-160, 162, 163], while these were systematically detected whenever screened.

2.2 C-reactive protein for discriminating bacterial from viral pathogens in the tropics

2.2.1 Research in context

C-reactive protein (CRP) was discovered in 1930 by William Tillett and Thomas Francis [71], who first described a nitrogenous sugar they named "*pneumococcus* Fraction C". In their report, this component was not found to be specific to pneumococcal infection and was detected in other Gram-positive cocci infections. In 1950, 20 years later, Paul Havens sought to measure but did not detect CRP among patients infected with viral hepatitis, and wondered if this was due to the pathogenesis of the viral infection [177]. In 1980, 50 years after the original discovery, Kardjito et al. were the first to measure CRP levels in the context of an infection in the tropics, on 90 Indonesian patients who were smear-positive for *Mycobacterium tuberculosis* [178].

Structural and pathophysiological analysis of CRP has been extended since then, describing a homopentameric structure with a production regulated at the transcriptional level by interleukine-6 (IL-6) [179]. More in detail, bacterial pathogens use particular pathogen-associated molecular patterns (PAMPs) including the recognition of peptidoglycans and lipopolysaccharides by receptors on immune cells, triggering the production of inflammatory cytokines including IL-6 [70]. IL-6 is not, however, exclusively stimulated by bacterial pathogens: viral PAMPs also activate inflammatory cytokines including IL-6, with subsequent elevated CRP-concentrations [180, 181]. In the same way, interleukine-1 (IL-1) and tumour necrosis factor alpha (TNF-α) induce CRP production in hepatocytes while being upregulated in case of a viral infection [182-184]. Therefore, CRP does not appear as a perfect biomarker of bacterial infection, the question being whether the degree of CRP elevation can be indicative of discriminating between bacterial and viral infections.

This second literature review includes all articles published in English language evaluating the concentrations and performance of CRP in distinguishing bacterial from viral infections both in Southeast Asia and sub-Saharan Africa. Details of the search strategy and studies selection process are provided in Appendix IV.

In this review, 162 articles were found, 59 (36%) were excluded because CRP was used to measure a chronic disease evolution (e.g. tuberculosis and human immunodeficiency virus) without any further microbiological investigations, 35 (22%) did not screen for any pathogen and 14 (9%) were case reports or included less than 50 participants. Out of 162 articles, 42 (26%) measured CRP concentrations and/or CRP performance for diagnosing at least one pathogen and were therefore included in this review. Only six studies were carried out between 1998 and 2008, compared with 36 (86%) between 2009 and 2019, suggesting a growing interest in measuring CRP in patients with a documented infection.

2.2.2 Study contexts

2.2.2.1 Southeast Asia *versus* sub-Saharan Africa

Studies from sub-Saharan Africa were the most prevalent (28 out 42), and a single study included countries from both sub-Saharan Africa and Southeast Asia, although CRP was evaluated in regard to pneumococcal colonisation without aiming to identify an active infection [185]. The distribution of studies was highly heterogeneous within regions: two-thirds (31/46) of sub-Saharan countries were not represented at all, while 8 of 28 studies in sub-Saharan Africa were based in South Africa. By contrast to sub-Saharan Africa, most Southeast Asian countries (8 out 11) with the exception of Myanmar, Brunei and Timor-Leste where represented in the Asian studies. Thailand was the country with the most frequent CRP assessments with four out 14 studies, although southern and western provinces were not covered [107, 110, 185, 186].

2.2.2.2 Urban *versus* rural areas

Rural areas were vastly under-represented. None of the eight South African studies was implemented outside of the main urban centres like Cape Town, Johannesburg or Durban [123, 124, 185, 187-191]. Paradoxically, over 19 out 57 million inhabitants of this country live in rural areas, with an upward trend over the last decades (https://www.indexmundi.com/facts/south-africa/rural-population). In Indonesia, study sites were limited to Java and only in urban areas [161, 192, 193], while

Cambodia was the only country in Southeast Asia to include a homogeneous distribution across urban and rural settings [21, 107, 194].

Out of the 42 studies, only five (12%) were based in a rural area, three in sub-Saharan Africa and two in Southeast Asia. This predominance of urban centres limits the generalisation of findings, because Africa and Asia concentrate 90% of the world's rural population [195]. Rural areas tend to be isolated and poor, explaining the scarcity of microbiological investigations on causes of fever and subsequent CRP assessments [94, 95, 196]. Furthermore, urban and rural regions vary by environmental exposure, lifestyle, diet, and health care access, and it is likely that the pathogen spectrum varies accordingly [197]. In rural areas for instance, deforestation and subsequent increased contacts between humans and wildlife are fuelling the burden of zoonotic diseases, in addition to intensive livestock production [133, 198-201].

Additionally, an individual's immune response towards one specific pathogen may be impacted by the global infectious burden in a given environment: healthy Ghanaians had a lower baseline CRP concentration than healthy Dutch despite repeated exposure to malaria, but a higher concentration during an acute phase response [93]. Other studies found that individuals with a high pathogen exposure (measured by household crowding and cleanliness, quality of water source, mode of waste disposal, and faecal exposure) tend to present a high baseline inflammatory level (e.g. CRP >3 mg/L) [94, 95]. CRP levels towards a particular pathogen may therefore depend on the environment an individual is living in.

2.2.2.3 Tertiary hospital *versus* primary health care

The level of care also impacts CRP concentrations: hospitalised patients present often with more severe comorbidities and symptoms and with particular pathogens, compared with ambulatory patients attending primary health care. In this review, only a third of studies included primary healthcare centres (15 out 42), limiting the evidence on CRP among non-severe outpatients. These few primary care-based studies included a large part of Southeast Asia with Cambodia, Lao PDR, Indonesia and Thailand, whereas five of twelve primary care-based studies in sub-Saharan Africa were centralised in South Africa, indicating that outpatient data on CRP are poorly distributed in this vast region.

Nevertheless, two-thirds (28 of 42) of the reviewed studies were based in a single site, which was a tertiary-care hospital in half of the cases (14 of 28), limiting the diversity of pathogens detected and the corresponding CRP measurements, as well as the generalisation to the whole country.

2.2.3 Clinical heterogeneity

Similar to causes of fever studies, inclusion criteria greatly varied across CRP assessments, altering their comparability. For instance, only 10 out 42 studies included all age categories. The immune system varies between individuals, and this variation increases by age particularly among young children or elderly individuals [202]. A low-grade inflammation is common among seniors due to a process named "inflamm-aging", which is for instance related to poor responsiveness to vaccines [203-205]. The link between a chronic inflammation and susceptibility to infection is increasingly described in the literature, although the pathophysiology remains unclear [206]. In addition to age, genetic, lifestyle and clinical factors also impact inflammatory response proteins [207]: in this review, only five (12%) studies enrolled febrile patients regardless of their clinical presentation whereas the vast majority of the studies restricted inclusion criteria to a particular syndrome such as pneumonia or meningitis, limiting the interpretation of CRP in febrile illness [21, 208-210].

In sub-Saharan Africa, CRP evaluations were often restricted to patients with HIV and/or tuberculosis without investigating any other pathogen. In South Africa, seven out of eight studies were limited to diagnosing tuberculosis using CRP, with a single exception on bacterial meningitis in children [191].

2.2.4 Microbiological heterogeneity

Details of microbiological methods used to evaluate the diagnostic accuracy of CRP in discriminating bacteria from viruses are presented in Tables 2.1 and 2.2.

Considering CRP measurements, most studies on *M. tuberculosis* provided a concentration, varying between 15.4 (outpatients) to 140 mg/L (inpatients). Corresponding performance was evaluated in seven out 11 studies, with the lowest sensitivity measured at 57% for an 80 mg/L threshold, and the greatest sensitivity at 98% for an 8 mg/L threshold. Specificity was at the lowest for a threshold set at

3 mg/L while it reached 96% for a 50 mg/L threshold. Approximately half of the studies (5 out 11) on *M. tuberculosis* calculated an area under the curve (AUC) to predict the presence of this bacterium, varying between 0.71-0.91 [123, 187-190]. Out of these five studies, one measured twelve inflammatory biomarkers, and CRP had a non-different performance than the one that was found to be the most discriminative analyte, namely haptoglobin (AUC [95% CI] 0.83 [0.73-0.94] and 0.85 [0.76-0.94], respectively) [188]. In this primary care-based study, CRP performance was similar to a seven-marker combination (sensitivity 75% *versus* 78%, specificity 85% *versus* 83%, respectively). All studies evaluating CRP performance in identifying *M. tuberculosis* were carried out among patients clinically suspected for tuberculosis, and not on all febrile patients. This is consistent with recent evaluations on outpatients with respiratory symptoms who first benefit from a clinical screening, increasing pre-test probabilities and subsequent CRP performance for ruling-out viral infections, including in sub-Saharan Africa [211].

Table 2-1 Microbiological methods by pathogen for CRP diagnostic accuracy evaluation

Pathogen	Country & Reference	Diagnostic methods	Limitations
Leptospira spp.	Indonesia [193]	MAT ≥1:320 on a single sample or four-fold rise MAT ≥1:80 from a deceased patient	Low threshold with risk of false positives
Campylobacter jejuni & coli	Thailand [186]	Serological methods in blood Microplate assay & PCR in stools	Indirect evidence for the presence of an acute infection
Salmonella enterica	Malaysia [83]	Blood & Stool cultures Serology: Widal & Typhidot tests	Poor accuracy of serological tests
	Cambodia [194]	Blood & Stool cultures Serology: White-Kauffmann scheme & WGS	Poor accuracy of serological tests
Streptococcus pneumoniae	The Gambia [212]	Blood, lung aspirates & CSF culture PCR	No control group for lung aspirates
Mycobacterium tuberculosis	Guinea-Bissau [213]	Microscopy	Methods not detailed
	South Africa [123]	Sputum: fluorescence microscopy Culture in liquid media or histology	
	South Africa [189]	Sputum: fluorescence microscopy Culture in liquid media	
	South Africa [190]	Sputum: fluorescence microscopy Culture in liquid media Xpert MTB/RIF assay Urine samples: TB-ELISA	
	South Africa [124]	Blood solid culture with niacin test	
	South Africa [188]	Saliva samples: MGIT methods; Ziehl-Nielsen & Capilia TB testing	
	South Africa [187]	Sputum: Ziehl-Nielsen & Auramine stains & Culture in liquid media Urine LAM assay	
	Tanzania [214]	Sputum: fluorescence microscopy Lowenstein-Jensen medium	
	The Gambia [215]	Sputum: Ziehl-Nielsen & Culture in liquid media	
	Uganda [216]	Sputum: fluorescence microscopy & GeneXpert MTB/RIF testing	
Ebola virus	Sierra Leone [217]	RT-PCR	CRP measured for severity
Hepatitis virus B	Nigeria [218]	Serology HBs Antigen	CRP compared between healthy, malaria and HVB cases
Measles virus	Zambia [219]	Haemagglutination antibody titre	Indirect evidence for an acute measles infection
Parvovirus B19	Nigeria [220]	Serology: solid-phase IgM ELISA	CRP measured for severity Poor assay performance for confirming an acute infection
Influenza virus	Singapore [221]	NP swabs: Gram strain & Culture & RT-PCR	Type A H1N1 No control group
	China & Vietnam [222]	Virus isolation RT-PCR Serology: four-time rise antibody titre	Type A H7N9
Cytomegalovirus	Zimbabwe [223]	RT-PCR	
	Indonesia [192]	Serology & PCR	

CRP: C-reactive protein; ELISA: enzyme-linked immunosorbent assay; LAM: Lipoarabinomannan; MAT: microscopic agglutination test; MTB/RIF: *Mycobacterium tuberculosis* complex and resistance to rifampicin; NP: nasopharyngeal; PCR: polymerase chain reaction; RT-PCR: Reverse-Transcriptase polymerase chain reaction; WGS: whole genome sequencing

Table 2-2 Microbiological methods by presentation for CRP diagnostic accuracy evaluation

Presentation	Country & Reference	Diagnostic methods	Limitations
Any febrile presentation	Cambodia [21]	Blood culture Nested-PCR: *Leptospira spp., O. tsutsugamushi, Rickettsia* and dengue virus Throat swabs: PCR influenza virus	Performance of CRP not described
	Cambodia, Lao PDR & Thailand [107]	Blood culture PCR & ELISA: *Leptospira spp., O. tsutsugamushi, Rickettsia,* dengue, JEV and Influenza virus	No performance of CRP by pathogen
	Zanzibar [210]	Blood: PCR on *Plasmodium*, *Rickettsia* spp., dengue virus, WNV, RVFV, chikungunya virus Urine: culture & *S. pneumoniae* RDT	Diagnosis of pneumonia based on X-ray No performance of CRP by pathogen
	Tanzania [209]	Blood & Urine culture	No performance of CRP by pathogen
	Thailand [110]	Blood culture PCR & IFA for rickettsiosis ELISA for dengue virus, JEV and *Leptospira* spp.	No performance of CRP by pathogen Imbalanced distribution of diagnostic tests
	Indonesia [161]	Blood & urine culture Serology: dengue virus, chikungunya, *R. typhi* and *Leptospira* spp. PCR for arbovirosis, rickettsiosis, leptospirosis & *S. enterica*	No pathogen description for blood culture No performance of CRP by pathogen
Meningitis	Philippines [82]	Blood & CSF cultures Latex agglutination test Counter-immuno-electrophoresis Enzyme immunoassay Viral culture	Unclear distinction between acute infection and past exposure for viral pathogens
	South Africa [191]	CSF Gram stain & culture RT-PCR for enterovirus	
	Malawi [128]	Blood & CSF culture Latex agglutination PCR: *S. pneumoniae, H. influenzae* & *N. meningitis*	Viruses not investigated
	Latin America & Angola [224]	CSF culture & PCR & Antigen detection: *S. pneumoniae, H. influenzae & N. meningitis*	CRP analysed for outcome Viruses not investigated
Pneumonia	Mozambique [129]	Blood culture NP aspirates PCR: RSV, Influenza virus, PIV, hMPV & adenovirus	No control group for NP aspirates
	Mozambique [130]	Blood culture	Viruses not investigated
	The Gambia [225]	Blood culture	Viruses not investigated CRP analysed for severity
	Togo [226]	Blood culture & NP aspirates & Multiplex PCR	No detail on each pathogen detected
	Asia & Africa [185]	NP swabs: Multiplex PCR & Culture & Serotyping Pleural fluid: pneumococcal antigen Blood culture	Restricted to *S. pneumoniae* colonisation
Bacteraemia	Vietnam [208]	Blood culture	Methods not described
	Tanzania [227]	Blood culture with chocolate agar	

CRP: C-reactive protein; CSF: cerebrospinal fluid; CNS: central nervous system; ELISA: enzyme-linked immunosorbent assay; IFA: immunofluorescence antibody; Lao PDR: Lao People's Democratic Republic; NP: nasopharyngeal; PCR: polymerase chain reaction; RT-PCR: Reverse-Transcriptase polymerase chain reaction; RDT: rapid diagnostic test.
H. influenzae: Haemophilus influenzae; O. tsutsugamushi: Orientia tsutsugamushi; N. meningitis: *Neisseria meningitis*; *R. typhi: Rickettsia typhi; S. enterica: Salmonella enterica; S. pneumoniae: Streptococcus pneumoniae;* PIV: parainfluenza virus; RSV: respiratory syncytial virus; hMPV: human metapneumovirus and JEV: Japanese encephalitis virus; WNV: West Nile virus; RVFV: Rift Valley fever virus.

Excluding *M. tuberculosis* (9 studies), around one-third (11 out 34) of studies were not analysing CRP by pathogen, but rather by clinical presentation and/or infection type (i.e. bacterial *versus* viral infection) [110, 128-130, 161, 191, 208-210, 225, 227]. Although the classification as either bacterial or viral infection relied on confirmed microbiological evidence, the absence of pathogen details limits the utility of these studies, since a bacteraemia may be due to a broad diversity of bacteria with corresponding CRP concentrations, depending on both the patient and their environment.

2.2.5 Tropical zoonotic pathogens

CRP was evaluated in tropical zoonoses in nine out 42 studies, and only in one of the 28 studies in sub-Saharan Africa, namely Ebola virus [217]. A 2019 systematic review of bacterial zoonoses in 29 African countries highlighted the lack of data on this continent, while 75% of the major disease outbreaks are due to this group of pathogens [228]. A 2018 literature review in East Africa reported a growing number of publications on zoonotic diseases over the last decade, with trypanosomiasis, brucellosis and Rift Valley Fever being the most commonly detected [229]. However, CRP evaluations were not found for these endemic pathogens in our review. In Southeast Asia, evidence on zoonotic pathogens was more available, but a single study provided the percentage of patients with CRP above certain concentrations for each zoonotic pathogen [107]. In this study, CRP performance was only calculated when grouping all zoonotic pathogens together.

Among the nine studies investigating zoonosis, the most common bacteria were *Orientia tsutsugamushi, Rickettsia typhi* and *Leptospira* spp., while the most frequent viral zoonoses were dengue and chikungunya viruses. CRP median for *O. tsutsugamushi* varied between 26 mg/L (outpatients) and 130 mg/L (inpatients), between 75 (outpatients and inpatients) and 103 mg/L (inpatients) for *R. typhi*, and between 19 (outpatients) to 150 mg/L (inpatients) for *Leptospira* spp. Considering viral zoonoses, CRP median varied between 8 (outpatients) and 18 mg/L (inpatients) in dengue virus, around 15 mg/L among outpatients with chikungunya virus. CRP performance was evaluated in a large spectrum of zoonotic pathogens among both children and adults at different levels of care in four countries, all in Southeast Asia [107, 110, 161]. However, none of these three studies provided CRP diagnostic performance for zoonotic bacteria specifically, mixing with pathogens

detected by blood culture. The first study estimated the AUROC between 0.78 (inpatients) and 0.89 (outpatients) with a sensitivity of 86% and specificity 67%; the second study measured a sensitivity of 88% and specificity 72%; the third one a sensitivity of 92%, specificity 73% and AUROC 0.91. Presented performances in these three studies corresponded to a 20 mg/L threshold. It is noticeable that sensitivities were consistently greater than specificities, indicating CRP to be more useful in identifying bacterial rather than ruling-out viral infections.

2.2.6 Bacterial blood stream infection

Considering studies assessing CRP diagnostic performance on pathogens detected by blood culture and not assessing zoonotic infections nor *Mycobacterium tuberculosis*, 12 studies were accounted for, with only one among outpatients [209]. Enterobacteriacea was the most common family isolated including *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella*. Among gram-positive bacteria, *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most reported.

CRP medians for bacterial blood stream infections varied between 22 (outpatients) to 178 mg/L (inpatients) [130, 209]. In terms of performance, AUC ranged between 0.60 (outpatients) to 0.88 (outpatients), with sensitivities between 50% (outpatients, threshold 20 mg/L) and 88% (inpatients, threshold 5 mg/L), and specificities between 70% (inpatients, threshold 5 mg/L) to 79% (outpatients, threshold 20 mg/L) for the presence of bacteraemia. The poorest CRP performance was found among six outpatients with a low CRP median (22 mg/L). In this study, only non-severe children were recruited in an outpatient department in Tanzania, with two out six blood culture-confirmed cases being *Salmonella enterica* Typhi, which may be detected among chronic carriers intermittently shedding bacteria for a prolonged period of time [209, 230]. Interpretation of these data is therefore challenging, and CRP may be of inferior accuracy for identifying bacterial but self-limiting infections. When considering hospitalised and more severe inpatients for *Salmonella*-confirmed samples, two studies measured CRP concentrations greater than in Tanzania, varying between 36-47 mg/L for *Salmonella enterica* Paratyphi A and Typhi respectively, with an AUC 0.64, a sensitivity of 68% and specificity of 58% using a 30 mg/L threshold [83, 194]. *Burkholderia pseudomallei* was only reported in three out twelve studies, all in Southeast Asia, and CRP was not separately analysed for this pathogen [107, 110,

208]. The scarcity of evidence on this bacterium contrasts with the fact that around 40% of the 1650,000 estimated cases worldwide are occurring in Southeast Asia with a 35% mortality rate [45, 231].

Regarding pneumococcal pneumonia, five studies found CRP to vary between 178-283 mg/L with an AUC between 0.65-0.87, sensitivity ranging between 73% (threshold 120 mg/L) and 95% (threshold 20 mg/L), and specificity between 45% (threshold 40 mg/L) and 77% (threshold 71 mg/L) [130, 185, 212, 225, 226]. The lowest sensitivity was 73% and corresponded to a high threshold of 120 mg/L among Gambian children with pneumococcal pneumonia [212]. In the context of resource-poor settings where safety and sensitivity should be preferred over specificity, lowering the CRP threshold from 120 to 40 mg/L would raise sensitivity from 73 to 87%, reducing the number of false negative pneumococcal cases.

Four studies focused on meningitis: three studies measured CRP by infection type and not by pathogen, with a median varying between 200-291 mg/L in bacterial meningitis *versus* 34-35 mg/L in viral meningitis [82, 128, 191]. The fourth study provided more details: for *Streptococcus pneumoniae*, CRP median was 160 mg/L; for *Haemophilus influenzae*, CRP median ranged between 160-161 mg/L; for *Neisseria meningitis*, CRP median varied between 134-189 mg/L [224]. Interestingly, CRP levels varied within the same study and for the same pathogen, depending on the country where the patient was recruited [224]. This suggests the role of an environment-individual interaction, through various exposures, genetic and lifestyle factors, resulting in host inflammatory differences towards a given organism [93-95, 197]. CRP performance was only calculated in two out four studies for detecting a bacterial meningitis: one measured an AUC (95% CI) 0.81 (0.73-0.89), and the second estimated a sensitivity of 94% and specificity 65% [82, 128].

2.2.7 Summary on performance of CRP in discriminating bacterial from viral pathogens

Evidence suggests a growing interest in evaluating CRP for the discrimination between bacterial and viral pathogens in Southeast Asia and sub-Saharan Africa. In this review, children and adults from primary, secondary and tertiary levels of care were included, which provides a comprehensive landscape of CRP performance for various age categories and levels of severity. Furthermore, CRP evaluations relied on high-quality microbiological methods in most cases, including blood culture,

molecular diagnostics, and serological tests with conservative thresholds. Considering the commonest thresholds selected by the studies reviewed (i.e. 10-40 mg/L), CRP performance was consistently defined by a high sensitivity and a moderate specificity in Southeast Asia and sub-Saharan Africa. Evaluations were carried out on a broad range of illnesses, including tuberculosis, tropical zoonotic infections, bacteraemia, pneumonia or meningitis.

This evidence may suggest a potential to recommend CRP-testing for guiding health workers in the management of febrile illness. In more detail however, evidence was unequally distributed: among the 46 sub-Saharan African countries, most CRP assessments were restricted to South Africa and among HIV-infected patients suspected for pulmonary tuberculosis, and only three studies investigated CRP on multiple pathogens regardless of the clinical presentation [209, 210, 227]. Out of these three studies -all paediatric and hospital-based- one was evaluating CRP performance using only six culture-confirmed positives cases; the second did not use blood culture, investigating five non-malarial pathogens using dried blood spot (DBS) only on a subset of patients, and CRP aimed to identify chest X-ray rather than microbiologically confirmed pneumonia; the third study exclusively assessed CRP among neonates suspected with septicaemia [209, 210, 227]. By contrast, four studies covering half of Southeast Asian countries were identified, including both children and adults from various levels of care, and CRP was evaluated on a broad range of pathogens microbiologically-confirmed and including blood stream infections and zoonosis [21, 107, 161, 208]. It is striking that very few studies provided the details of their microbiological findings with corresponding CRP concentrations, which limits the comparability between CRP evaluations [21, 107, 110, 161, 209, 227].

Considering tropical zoonosis, this review highlighted additional gaps, particularly in sub-Saharan Africa where a single study provided CRP levels for one unique organism, Ebola virus, while endemic zoonotic pathogens represent the majority of zoonosis in sub-Saharan Africa [217, 229]. In Southeast Asia, three studies calculated CRP performance in distinguishing bacterial from viral zoonosis, but none of them assessed each zoonotic pathogen separately. Zoonosis is estimated to account for around 60% of all communicable diseases among humans in the world, with rural areas being at great risk of exposure, due to intensive deforestation and livestock production [133, 198-201, 232]. Extending CRP evaluations on zoonotic pathogens is therefore warranted.

Nonetheless, CRP was reported to show the greatest performance among patients who were clinically screened for pulmonary tuberculosis [123, 124, 187-190, 213-216, 233]. The lowest performance was found among non-severe outpatients in Tanzania, where CRP median was measured at 22 mg/L in case of positive blood culture [209]. Despite the low number of culture-confirmed cases limiting the interpretation (n=6), it is possible that CRP may be of a lesser utility when aiming to discriminate between bacterial and viral pathogens among patients with a self-limiting infection.

Finally, CRP showed a moderate specificity for the most frequent thresholds selected (i.e. 10-40 mg/L), implying an overestimate of bacterial infections: restricting CRP-testing to patients preliminarily classified at risk of bacterial infection may enhance CRP specificity by reducing the proportion of false positives. In this scenario, CRP may be used as a rule-in for a serious bacterial infection, and the impact could be strengthened by selecting a high threshold (i.e. ≥80 mg/L). In low-level care and especially in resource-poor settings, clinical management broadly varies between prescribers [34, 35, 234]. In this context, safely ruling-out a potentially serious bacterial infection based on a brief physical examination done by a minimally trained healthcare worker may be unrealistic. Additionally, population living in poverty tend to be hard-to-reach with challenging follow-up. A strategy testing all attending febrile patients may therefore ensure further safety, especially if using a low CRP threshold (i.e. < 20 mg/L). Correspondingly, CRP was shown to be highly sensitive, suggesting to be a safe tool for ruling out serious bacterial infections.

2.3 Impact of C-reactive protein-testing on antibiotic prescription in primary care

2.3.1 Research in context

Primary care represents the front gate of a health system that facilitates equitable distribution of broad health services among the general population [235, 236]. The higher the ratio of both primary care settings and health workers to population, the lower all-cause mortality rate [237-239]. A case-control study showed that the presence of primary health service in one of the poorest states of Mexico significantly reduced mortality among infants with an acute respiratory tract infection (ARI), highlighting the importance of access to the most vulnerable populations [240]. Antibiotic appropriateness was also a significant factor affecting infant outcomes in this study, as well as trust in the public health system, which relates to another study from the same research group, where 80% of patients dying at home from ARI or acute diarrhoea sought care through private health providers [241].

Identifying patients who need an antibiotic remains a challenge in all primary care: a US review including 14 primary care units (PCUs) estimated that upper respiratory tract infection (URTI) was the most frequent diagnosis for which a visit at the health centre was not necessary, suggesting that most primary care patients are not severe and may not require any antibiotic management [242]. Most URTIs are indeed self-limiting with a viral aetiology and should exceptionally be treated by antibiotic: no bacterium is known to cause common cold while it represents the largest component of URTI [243]. Despite being an exception in primary care, URTIs can be caused by a bacterium, with potentially severe outcomes [244]. Prescribers therefore face the challenge to identify these rare bacterial infections without obvious clinical symptoms. This dilemma may explain why antibiotic prescription is common for URTIs both in children and adults [245, 246], with no less than three quarters of all antibiotics estimated to be prescribed in low-level care [247]. In the United Kingdom (UK), 36% of common colds, 40% of sore throat, 70% of otitis media and 90% of sinusitis were reported to be treated by antibiotics in 2016, while these are generally self-limiting infections [244]. In 2017, a trial gathering 117 general practitioners (GPs) and 16,535 patients in four South American countries measured antibiotic

prescription at 71.6% in case of acute bronchitis and 94.8% in acute otitis media [248]. In 2018, 47% of all acute patients attending northern Thai PCUs were prescribed an antibiotic, and this proportion rose to 83% and 89% in cases of acute sinusitis and pharyngitis, respectively [249].

Not only respiratory infections are driving antibiotic prescription in primary care: in the 2018 northern Thai study, two-thirds of patients presenting with a gastro-enteritis or colitis had an antibiotic prescribed, although most cases are viral and/or self-limiting [249]. It is striking that 13.8% of those who did not present with any fever, history of fever, nor signs of infection had an antibiotic prescribed in that study. In India, fever was reported to be the most common symptom among over 200,000 primary care patients across 880 cities and towns in 2011, and gastrointestinal presentation was the second most frequent cause of attendance among under 30-year-old patients [250, 251]. Rotavirus is one of the key pathogens causing non-severe diarrhoea among all ages, and an oral rehydration solution is the recommended treatment [252, 253], however, antibiotic levels remain high: in New Delhi, 43% of diarrhoeal episodes were treated by at least one antibiotic in a 2007-2008 community-based study; and in Nigeria, it reached 85% among under-5 children attending two-large health centres with watery diarrhoea in 2016-2017 [254, 255].

Antibiotic prescription is not only high in primary care, but also inconsistent: in Cameroon, wide variations were reported between prescribers from 10.7 to 58.7%, while fluctuating from 43 to 69% between public and private clinics in India [254, 256]. Acute pharyngitis can be due to group A *Streptococcus* (GAS) in approximately 5-15% among adults and 20-30% among children, with rare but severe complications in absence of antibiotic treatment [257]. A study comparing prescribing practices between Europe and South America including 457 primary care prescribers and 6,394 sore throat patients found substantial variations from 38-88%, and also within countries, some health workers systematically prescribing antibiotics while others never did [35]. This study reported a substantial heterogeneity across sites: all GPs from Denmark and Sweden had access to a Strep A test but over 80% of those in Russia and Argentina did not; the ratio of health worker to patient was the lowest in Argentina and tripled in Sweden; one out of 614 (0.1%) Danish patients asked for an antibiotic while 9% of Russians did. Authors linked such variations to differences in patterns of thinking, training and policy environment, as well as in staff and diagnostic tool supply. This heterogeneity is global: among

12 Asian countries, antibiotic prescription for all outpatients widely ranged between 12-62%, and between 43-94% among those with an URTI [34].

Additional constraints should be considered considering the context of LMICs: the average patient-provider interaction lasts less than 1 minute according to a WHO report gathering primary care data from 1990 to 2006, while a systematic literature review covering 67 countries found that half of patients spend 5 minutes or less with their primary health workers [34, 234]. In this latter study, the time of consultation varied widely between 48 seconds in Bangladesh and 22.5 minutes in Sweden, while the WHO has anticipated a global shortage of 18 million health workers by 2030 [258].

2.3.2 Point-of-care testing & C-reactive protein

Regarding the challenges identified in primary healthcare, improving patient management may require multiple layers of interventions from the highest levels of political sphere supporting antibiotic stewardship programmes to health worker's daily practice identifying patients who need an antibiotic.

Among the tools available to guide patient management at the time of the consultation, POCT can inform health worker's decisions by improving diagnostic pathways [55]. In addition to clinical effectiveness, POCT must be cost-effective: WHO recommends applying the ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end-users) prior to POCT integration in routine care.

Since its discovery in 1930, several evaluations of CRP have been carried out with the objective to improve the identification of primary care patients who actually need an antibiotic. Cost-effectiveness analyses have already shown CRP-testing to be beneficial in primary care, including in resource-poor settings [97, 103, 259].

Reviewing the literature on the impact of CRP POCT on antibiotic prescription in primary care, 84 articles were initially identified. We included publications in English language from high- and low-to-middle-income countries, and the search strategy was completed with articles from reviews and meta-analyses [79, 260-265]. Out of the seven reviews identified, one was not accessible [261]. The six remaining included a total of 18 studies with 45,151 primary care patients, and 17 out these 18 studies were based in Europe and/or evaluated CRP in respiratory presentations exclusively. In our review, we

found a total of 86 studies: 38 (44%) were kept in the analysis, 17 (20%) were excluded because these were not assessing the impact of CRP on antibiotic prescription, and 13 (15%) were either case reports, qualitative or cost-effectiveness analyses (Appendix V). Among the 38 studies reviewed, 80% were published over the last ten years (2009-2019) while first publications appeared in 1995, suggesting a growing interest in evaluating CRP for rationalising antibiotic prescription in primary care.

2.3.3 Geographical heterogeneity

Asia is the epicentre of antimicrobial resistance (AMR) globally [43], but only two out of the 38 studies reviewed were implemented in this densely populated region, excluding major contributors of antibiotic use such as India, Bangladesh or China [266-268]. This maldistribution extended to LMICs in general: in Africa only three studies were identified (in Ethiopia and Tanzania), while countries with an acknowledged antibiotic overuse such as South Africa, where 78% of patients attending public clinics receive antibiotics, were not included [269]. It is concerning that the majority of African countries have not adopted antibiotic regulation in their national policy, with a very few number of countries enrolled in the Global Antimicrobial Resistance Surveillance System (https://www.who.int/glass/country-participation/glass-map-1200px.jpg). In South and Central America, Argentina was the only country where CRP-testing was evaluated, with a total of 143 patients and only among those with an acute exacerbation of chronic obstructive pulmonary disease [270].

Within high-income countries, disparities were also prevalent: although 82% of studies were implemented in Europe, countries with high rates of antibiotic prescription such as Greece, Romania, or Portugal never reported CRP POCT evaluations [271]. It is striking that neither Australia, Canada nor the USA were represented in this primary care-based review, and this geographical imbalance limits potential generalisation of CRP POCT: antibiotic prescription is based on health workers' behaviour, which depends on the local context. For Bourdieu, 'habitus' is a system of ingrained habits related to an unconscious interaction between the social world and the individual: it implies that some components of an individuals' decision are not rational, with an unconscious mechanism [272]. Consistently changing human behaviour (i.e. antibiotic prescription) through a specific intervention (i.e. adhering to a CRP test) without taking the geographical and subsequent socio-cultural particularities into account

may be unrealistic. A qualitative analysis of the CRP POCT impact across three different countries in Southeast Asia has shown a wide range of risk perception towards infectious diseases, policy environment, local health system structures and patient preferences depending on their conception of illness, health workers and drugs [273].

Altogether, this suggests the necessity to integrate the specificities of a given context in the quantitative measurement of an intervention. As an illustration, evaluations of CRP POCT using similar methodologies showed various effects on antibiotic prescribing in this review, both between and within studies. In Vietnam, a randomised controlled trial gathering over 2,000 primary care adults and children with respiratory symptoms showed a significant heterogeneity between the 10 sites [274]. This suggests a cautious interpretation when generalising study findings to a broader environment.

2.3.4 Methodological evaluations

In our review, most studies included more than one site to evaluate CRP POCT, with an equal distribution of observational and randomised controlled trials. In terms of design, 34 out 38 studies were prospective and 19 were randomised controlled trials (RCTs), implying a high-level of evidence due to the use of a control group. A few comments: in case of individual randomisation, CRP POCT may be of a lower impact on antibiotic prescription because health workers are both controls and exposed to the intervention. As most patients tend to present with non-severe symptoms, corresponding inflammatory levels are generally low, and it is likely that prescribers -advised to withhold antibiotics in these cases- gain awareness and stop prescribing to controls with a non-severe presentation. Trials using health centre as a unit of randomisation are not undermined by this dilution effect. Another bias of individually randomised trials is due to a preferred enrolment of the least severe patients (without clear criteria), in order to reduce potential risks of antibiotic withhold (i.e. selection bias). Cluster randomised trials suffer from potential biases as well: sample size calculation needs to be inflated compared with individual randomisation methods as the data is reduced to one observation per cluster; clusters may not be comparable in terms of patients and exposure (i.e. recruitment bias) although this can be quantified by the intra-cluster correlation coefficient (ICC) [275].

Prospective observational studies are not considered gold standard, but offer the advantage of being more inclusive: the majority of the 20 RCTs used several exclusion criteria such as severity, while this latter criterion was dependent on the health worker's judgment without a clear definition. Most patients with an impaired immune status were also excluded from the reviewed RCTs, although a systematic review and meta-analysis gathering 12 studies from high- and low- to middle income countries demonstrated CRP to be a reliable marker for identifying patients in need of an antibiotic even in case of severely immunocompromised individuals [276].

Only five studies used a retrospective design for assessing the impact of CRP POCT on antibiotic prescription [277-281]. By contrast to prospective evaluations undermined by the Hawthorne effect, prescribing behaviour is not affected by the observer effect during retrospective studies, and these included a large sample size (over 168,000 patients). However, all of them were implemented in Europe and only among patients with a respiratory presentation. It is unclear if children were included in these retrospective analyses, but the median age was ranging from 27 to 55-year-old, suggesting that most records corresponded to adult outpatients. Similarly, only 10 out of the 34 prospective studies clearly indicated a paediatric recruitment [99, 100, 211, 274, 282-287]. The immune system evolves alongside age, and it has been demonstrated that CRP concentration varies accordingly [203-205]. Evaluations including both children and adults for various CRP thresholds are likely to capture such immune variations: in this review however, only one study enrolled all patients aged or older than one-year-old, assessing different thresholds, which refines prescribing decision by tailoring CRP POCT cut-off [274].

The impact of CRP POCT may be biased by further methodological limitations: sample sizes are generally calculated for reducing prescription by a certain proportion, which is systematically overestimated. Schot et al., for instance, anticipated a 20% reduction and powered their population accordingly but only obtained a (non-significant) 9% reduction [282]. The original hypothesis assumed a baseline prescription rate of 70%, whereas it was actually measured at 40% in the control group, worsening the under-calculation of the sample size. Finally, this study prematurely ended due to slow recruitment and did not reach the planned sample size, similarly to Melbye et al, due to health workers' lack of interest [116]. Another RCT did not inform about its sample size strategy nor the objective of

antibiotic reduction, weakening the interpretation of the results [287]. In this trial, health workers seemed to only follow CRP results for high concentrations (OR 1.1 per unit increase, p-value <0.0001), suggesting a preferential use to rule-in patients with high CRP concentrations for safety reasons rather than to consistently integrate CRP in their clinical judgment.

2.3.5 Additional interventions

Additional interventions were evaluated concomitantly to CRP POCT in this review: information and communication trainings were independently reducing antibiotic prescription, while the combination of the test with such interventions further impacted prescribing behaviour [114, 117, 119, 288]. Another factor to be described is the policy environment: in Thailand, a national policy set a 20% prescription objective among primary care patients with a respiratory infection or an acute diarrhoea, including financial incentives to reach this target since 2016 [289]. As an illustration, antibiotic prescription was around 47% in 32 PCUs in Chiang Rai in 2015-2016, while it was measured at 31.8% in the control group from an RCT implemented in similar settings in 2016-2017 [100]. Similarly, a 55 country analysis has shown a 20-30% reduction among patients with URTI or acute diarrhoea in case of an adequate policy environment (having a national Ministry of Health unit on promoting rational use of medicines, a national drug information centre and provincial and hospital drugs and therapeutics committees), which implies not only educational but also policy managerial components [290].

At point-of-care, reinforcing clinical training for primary healthcare following international standards may represent a relevant option, particularly in resource-poor settings: in Uganda, a study identified 22 different organisations in charge of community health workers (CHWs) training without any standardised methods [291]. A literature review on 35 studies -all in LMICs- reported a high heterogeneity in type, frequency, duration and focus of training delivery, and a majority of these training programmes had a narrow geographic concentration, suggesting even greater gaps in health worker competences and distribution [292].

Last, Keitel et al. demonstrated both efficacy and safety of antibiotic reduction when CRP is combined with additional diagnostics (including oximeter, haemoglobinometer, procalcitonin, CRP

POCT and glucometer) combined in an electronic algorithm (e-POCT) with health workers training and supervision [99, 211]. It is noticeable that antibiotic prescription was reduced by more than 80% compared with usual care (p-value <0.001), and by 43% compared to ALMANACH (p-value 0.005). If the combination of CRP POCT with additional interventions seems to maximise antibiotic reduction, a few comments are warranted: scaling-up a whole point-of-care diagnostic platform may not be realistic regarding the allocated resources and time in primary care (e.g. 48 seconds per consultation in Bangladesh) [234]; Tanzanian prescribers were not allowed to overrule the e-POCT guidance while it is unlikely that health workers would strictly comply to any prescriptive rule and discard their clinical judgment. Emphasising this opposition between efficacy and effectiveness, Tanzanian prescription rates in routine care were unusually high compared to those measured in European RCTs (95% *versus* 22-58.9%, respectively), also suggesting an increased impact of POCT in settings where prescriptions are the highest [113, 286]. As an illustration, testing for CRP showed no effect in Sweden and Norway where the routine prescription was 36%, whereas there was a marked antibiotic reduction in Wales where baseline prescription was 70% in the same study [293].

In our review, the greatest antibiotic reductions were achieved in studies not relying on CRP POCT as a single intervention, but combining it in a set of measures [99, 114, 211, 270, 288, 294-296]. Most joined interventions were additional medical tests including chest X-ray or white blood cell count, as well as training in communication skills. This latter intervention was often assessed separately without CRP POCT, showing a significant impact on prescribing behaviour [114, 117, 119, 288], although safety was only assessed in one study [119]. Considering studies evaluating CRP POCT as a single intervention: nine out of 22 (41%) quantified a substantial antibiotic reduction (i.e. over 20 percentage-points) [113, 114, 117, 288, 293, 297-300]; seven (25%) described a modest but significant antibiotic reduction (i.e. around 10 percentage-points) [100, 115, 119, 274, 281, 301, 302]; four (15%) showed no effect [116, 282, 284, 287]; and two reported a non-significant increase of antibiotic prescription [285, 286]. A few comments: studies with an over 20 percentage-point reduction recorded high baseline prescription levels ranging between 54-92%; among the four studies reporting no significant antibiotic reduction, two were under-powered and stopped recruitment due to a slow recruitment [116, 282] and two noted a low adherence to CRP results [116, 287]; among the two studies

reporting a non-significant antibiotic increase, one was powered for a qualitative assessment and not for quantifying the CRP POCT effect on antibiotic prescription with only 54 patients included, while the other one was under-powered because of both an overestimate in baseline prescription levels and a premature enrolment interruption [285, 286]. In this latter study, 0-6-year-old Norwegian children with a fever or respiratory symptoms were prescribed more antibiotics when tested with CRP prior to any clinical assessment, compared with those not tested (36/138 [26%] *versus* 57/259 [22%], respectively, p-value 0.361). The authors suggested restricting CRP POCT on a subgroup of patients selected after a medical consultation, especially in countries like Norway where prescriptions levels are already low. In Tanzania where antibiotic prescription was around 95% in routine care, Keitel et al. also recommended not testing all febrile patients with CRP but only those with a "reasonable" pre-test probability of bacterial infection [211]. CRP being moderately specific, ruling-out false positives by a physical examination may improve CRP-testing performance, especially if using a high threshold (e.g. 80 mg/L).

Some precautionary comments have to be made: CRP POCT has never been recommended to be used as a single diagnostic tool without concomitant clinical evaluation; none of the 38 articles reviewed -including those from Norway and Tanzania- ever assessed the impact nor safety of CRP POCT restricted to patients classified at risk after a medical examination; excluding patients from CRP POCT based on clinical judgment may be unsafe regarding its variability, even when clear clinical criteria are defined [35]. Altogether, this warrants evidence comparing CRP-testing in all febrile *versus* those at risk of a treatable infection, both in terms of antibiotic prescription and safety, under various conditions of resources, prescription levels and populations.

2.3.6 C-reactive protein threshold & Safety

The most common thresholds reviewed were 5, 10, 20 and 40 mg/L, although no study compared different threshold performances. Nonetheless, CRP medians were varying between 9-22.5 mg/L which may be related to the absence of severe patients across these 38 reviewed studies in primary care, suggesting that future threshold evaluations may be relevant around or below 40 mg/L in this particular environment [100, 282].

The reviewed studies primarily aimed to measure the impact of CRP POCT on antibiotic prescription, and only one evaluated safety as the primary outcome with an adequate sample size [211]. Keitel et al. were therefore powered to detect a 3% difference in clinical outcome at 7 days of enrolment between the control group and the e-POCT, and relied on a physical examination with pre-defined symptoms to evaluate safety (risk ratio RR 0.60 [0.37-0.98]). By contrast, Schot et al. measured the proportion of re-consultations between the controls and the CRP group within a 3-month follow-up to ascertain safety (29 and 16%, respectively, odds ratio OR 0.61 [0.32-1.17]), but the sample size was calculated to reach a 20% antibiotic reduction rather than the absence of differences in adverse events, and patients with persisting or worsening symptoms may have consulted elsewhere [282].

Demonstrating safety is challenging even with appropriate sample size: in Tanzania, most primary care children had CRP concentrations below 40 mg/L, and this study was not powered for ascertaining patient safety in case of high levels [211]. It is therefore debatable whether using such high threshold (i.e. 80 mg/L) for such minor antibiotic reduction (16 patients with a CRP concentration between 40-80 mg/L being prescribed) was safe. Besides, antibiotic prescription rate reduced to 2.3% using a threshold of 80 mg/L, and one may wonder if the proportion of children with a bacterial infection was actually greater, especially among those presenting with lower respiratory tract infection (LRTI). A 10-year meta-analysis and retrospective cohort analysis of ARI in ambulatory clinics have estimated that 27.4% of children had a bacterial infection [303]. Whether the reduction obtained by Keitel et al. in Tanzania was safe remains difficult to ascertain, and apart from that study, none of the 14 studies reporting CRP POCT safety was adequately powered to ascertain it [100, 113-116, 119, 274, 282, 288, 294, 295, 299, 301, 304]. None of these 14 studies exclusively relied on a systematic clinical examination to ascertain patient recovery, and instead allowed for phone interviews when clinical examination was not possible, or used criteria such as frequency of re-consultations or delay in collecting antibiotics after prescription.

Additionally, health workers prescribed more antibiotics to patients with high CRP levels, indicating that testing for CRP may not only be considered to reduce antibiotic prescriptions but also to cover patients with high inflammatory concentrations. Despite a moderate specificity implying a risk for over-treatment, significant antibiotic reductions were still obtained: for instance, Bjerrum et al.

reported an antibiotic coverage of 80% among patients with a CRP concentration above 25 mg/L, which may be an excessive proportion, although overall antibiotic reduction was still substantial by 19% [297]. Whether a higher threshold would have reduced antibiotic prescription further without affecting safety remains unknown, and future studies sufficiently powered to assess patient safety for high CRP concentrations and comparing various thresholds are warranted.

2.3.7 Summary on antibiotic prescription in primary care

This review aimed to identify evidence and limitations of the impact of CRP POCT on antibiotic prescription in primary care.

Evidence was of a high-level of quality, with a broad diversity of evaluations including the use of randomised-controlled trials both individually-based and by cluster, as well as prospective observational studies allowing a broader inclusion of patients and retrospective studies providing a more pragmatic analysis without any research environment.

This review also highlighted the impact of additional interventions to CRP POCT on antibiotic prescription, such as patient information and communication skills training [114, 115, 117, 273, 288].

A major comment deals with heterogeneity, with countries such as China or India with vast populations and where antibiotic prescription is the greatest and where no evaluation was carried out [266, 268, 271]. Heterogeneity also encompasses prescribers, with substantial prescribing variations for the same presentation, even in the same facility. This latter aspect has to be linked to the importance of the context in prescribing behaviours. For instance, the policy environment including medicine regulation, guidelines and training programmes is inconsistently implemented in LMICs, where antibiotic prescription is common [305]. Furthermore, patient presentation is not specific enough to solely rely on health worker's judgment, and many subjective factors from both health workers and patients interfere with the decision to prescribe or not an antibiotic. Rationalising patient management in case of fever therefore appears as a priority. With this objective in mind, point-of-care diagnostic tools to consistently identify patients who need an antibiotic may be of relevance.

Children were often excluded from this evaluation while they represent a substantial driver of antibiotic prescription in primary care [256, 306-308]. Among the four studies identified in LMICs,

only one included children and adults regardless of clinical presentation and compared different thresholds. The three remaining studies were restricted to children with respiratory symptoms [99, 211, 274]. Although these represent the most common reason for first-contact medical care, fever and digestive symptoms are also common and act as a major contributor to antibiotic prescription. CRP POCT evaluations should therefore include broader febrile illnesses, bearing in mind that testing all febrile patients without any concurrent clinical evaluation is not recommended.

Finally, future studies are warranted to compare different CRP thresholds, with particular attention to the local healthcare constraints for better weighing the attractive prospect of major antibiotic reduction with patient safety, particularly among patients living in hard-to-reach areas.

Chapter 3

METHODS

3 METHODS

3.1 The CRP Study – Sites & Population

The CRP Study was a multi-centre open label randomised-controlled trial, with the primary aim of evaluating whether C-reactive protein (CRP) point-of-care test (POCT) could aid health workers in the discrimination of patients who need an antibiotic, as well as identifying the key pathogens causing acute fever in Thailand and Myanmar. We aimed to extend CRP POCT evaluation to all acutely febrile children and adults attending primary care.

In Thailand the study sites were selected from the primary care units (PCUs) in Chiang Rai with the intention of ensuring diversity in terms of the rural/urban environment. Two PCUs were initially included with four additional sites opened due to slow recruitment. Mueang (or township) Chiang Rai is located within Chiang Rai, the northernmost province bordering Myanmar and Lao People's Democratic Republic (PDR). It is defined by a tropical climate, and the population is majority Thai, with approximately 15% ethnic minorities and hill-tribes. Specimens were collected from six PCUs located within a 30-kilometre radius of Chiang Rai city centre, covering rural and peri-urban as well as mountainous and plateau areas.

In Myanmar, the study was conducted in Hlaing Tha Yar, the poorest township of Yangon, in three Medical Action Myanmar (MAM) clinics and in one adjacent hospital outpatient department [309]. MAM clinics are located in areas where a large proportion of the population cannot afford to pay for their basic health needs. The clinics provide a mix of services including mother and child care, reproductive health, human immunodeficiency virus (HIV) and tuberculosis (TB) management. Each clinic routinely manages around 50 patients a day. Hlaing Tha Yar, is a peri-urban township on the west side of Yangon (formerly capital city of Myanmar). It is defined by a tropical monsoon climate. The township has the highest rates of disease related to hygiene and environmental conditions (e.g. diarrhoea, dysentery, and tuberculosis) in Yangon [309]. Four sites were included: three primary care clinics and one outpatient department from a public hospital. Further details are shown in Table 3.1.

Table 3-1 The CRP Study: sites & population

	Chiang Rai – Thailand	**Hlaing Tha Yar – Myanmar**
Sites	6 public primary care units	3 primary care clinics 1 outpatient department (government hospital)
Location	Rural and peri-urban settings within a 30-kilometer radius of Chiangrai city centre	Slum area, peri-urban township on the west side of Yangon
Healthcare provider	2-3 registered nurses per site	2-5 medical doctors per site
Access fees	Universal health coverage	Free
Population	Thai community, 15% ethnic minorities	Mainly Burmese community
Investigations routinely available	Finger-prick blood glucose test	Rapid test for malaria
Malaria transmission	0-0.1 cases per 1,000 population	0-0.1 cases per 1,000 population

In the trial, we recruited all patients aged ≥1 year with a documented fever (defined as a tympanic temperature of >37.5˚C, according to World Health Organisation [WHO] standards) or a chief complaint of fever within the last 14 days [310]. Those with previous antibiotic intake and co-morbidities other than malignancies were also enrolled. We did however exclude severe patients who should be referred to hospital, defined as either alteration of consciousness, presence of seizure or where oral administration of medication was not possible; patients with a positive malaria test; the main reason for attending being trauma and/or injury; suspicion of either tuberculosis, urinary tract infection or local skin/dental abscess/infection; patients with any symptom present for more than 14 days; any bleeding; or inability to comply with the follow-up visit at Day 5.

3.2 The CRP Study – Methods for measuring the impact on antibiotic prescription

3.2.1 Randomisation procedure

The CRP Study groups consisted of the following:

- Intervention Groups A and B: CRP was measured by study staff onsite and the result was communicated to the healthcare worker as "low-CRP" or "high-CRP" using a 20 mg/L threshold (Group A) or a 40 mg/L threshold (Group B).
- Control Group C: The healthcare worker was asked to manage febrile patients as standard care.

Patients were randomised at a 1:1:1 ratio to either one of the two intervention groups or the control group stratified by country (Thailand and Myanmar) and age category (children and adults, defined as ≥12 years of age). Computer-based individual randomisation was done at the Mahidol-Oxford Tropical Medicine Research Unit (MORU) by the clinical trial statistician using the *ralloc* command in STATA version 14. Randomisation envelopes were numbered, sealed, opaque, and contained the study group the patient was allocated to. These envelopes were then opened sequentially on site by the study staff after patients were screened and included.

Both healthcare workers and patients were blinded to allocation between the two intervention groups but by design were aware of allocation to the control group. The study staff could not be masked to patient allocation between all groups and were not involved in the patients' routine medical examination nor antibiotic prescription.

3.2.2 Threshold selection

The study design included two intervention groups with CRP thresholds of 20 mg/L and 40 mg/L to guide antibiotic prescription, and one control group representing standard care without CRP POCT. These two thresholds were chosen according to previous evidence on CRP concentrations in febrile patients [311, 312]. Given the potential influence of the geographical location on variation of inflammatory levels due to particular pathogen exposure, we particularly targeted studies from

Southeast Asia: a first study evaluated CRP performance in specimens collected throughout Southeast Asian hospitals and clinics, showing both bacterial zoonoses and bacteraemia with CRP medians above 20 mg/L, with a sensitivity and specificity of 86% and 67% respectively [107]. A second study recruiting hospitalised adults in Chiang Rai identified 20 mg/L and 40 mg/L as candidate thresholds for detecting bacterial infections (sensitivity of 92% and 86%, respectively) [110]. Pregnant women were not excluded from our study criteria, as a study targeting this specific population showed CRP median to be under 20 mg/L in microbiologically-confirmed viral infections, while it was above 40 mg/L in bacterial infections in Lao PDR [313].

3.2.3 Study information

Prior to the study launching, we informed the primary care staff in local language about CRP performance in helping identify patients in needs of an antibiotic. The information included how CRP results should be used, the importance of clinical judgment, and was delivered based on previous models developed for CRP testing, adapted to the context in Thai and Myanmar settings [314]. Healthcare workers were encouraged to limit their antibiotic prescriptions for patients without clear danger signs and low CRP concentrations, while for patients with high CRP the guidance was to favour antibiotic prescription. They were reminded that CRP performance was not perfectly accurate for ruling out patients who do not require an antibiotic, emphasising the importance of their clinical judgment. A refresher session was implemented around the mid-point of study recruitment.

Information about the risks and benefits of participating in the study was provided to potential participants by the research team in local language, and informed consent was obtained before performing any study intervention. Patients under 18 years of age were asked to give assent depending on their age and local ethical regulations, and a parent or guardian was required to provide a written approval on the Informed Consent Form (ICF). In the case of illiteracy, a witness was asked to sign the ICF in order to ensure the participant gave an informed verbal consent to join the study.

3.2.4 Patient management

Patients randomised in one of the two intervention groups had a capillary blood specimen taken for CRP-testing in the facility by the research team. Capillary blood specimen was then tested using a CRP reader (NycoCard II Reader, Axis Shield, Norway). The Nycocard Reader II is based on an immunometric principle, and has been validated in a broad range of patients and healthcare facilities [124, 315-318]. Measurement range in whole blood is 8-200 mg/L, and each test costs around USD 4, depending on the number of tests to be ordered. This test integrates constraints in terms of both costs and access, and therefore comply with the ASSURED criteria [319]. This reader is, however, characterised by some limitations that need to be taken into account prior to usage: its actual implementation is limited in the context of tropical and low middle income countries (LMIC); reagents must be stored in a fridge at low temperature with expiry date limited to 6 weeks, coming down to 1 hour at room temperature; very low or high CRP values are imperfectly correlated to standard laboratory methods; CRP quantification assumes a haematocrit at 0.40 in whole blood, which is uncommon in septic patients; several steps preparing reagents are needed before measuring CRP; quality assessment is missing since storing CRP results is not possible [315, 318, 320].

A brief educational video on the importance of antibiotics, the risk of overuse, as well as on CRP utility was shown to patients randomised in the intervention groups, in order to ensure patients' understanding of the study. Following testing, the research team provided the patient a card indicating whether their CRP levels were high or low in relation to their intervention group, and then referred to the healthcare worker. Patients randomised in the control group had a venous blood specimen taken, stored at +4˚C, and retrospectively tested for CRP concentrations. All patients then went through a routine medical examination by the primary care health worker, who made the decision whether to prescribe an antibiotic or not. Demographic and clinical information was collected by the research team on a Case Report Form (CRF).

Two follow-up consultations were organised at Day 5 (allowable range 4-7) and Day 14 (allowable range 12-16) after enrolment by face-to-face appointment with the research team. Where patients were not able to attend in person, a structured telephone interview was carried out instead. All patients were tested for CRP at the first follow-up visit (Day 5) on site by the research team to help

measure clinical recovery regardless of their randomisation group, and healthcare workers were only informed about CRP levels if these were ≥50 mg/L in children, and ≥100 mg/L in adults. We compensated patients for participation and covered travel expenses at enrolment and on each of the follow-up visits that they attended in person.

3.2.5 Outcome

Our primary outcome was the prescription of any antibiotic at the facility from Day 0 up to Day 5, measured in total, above and below the CRP thresholds of 20 mg/L and 40 mg/L. Secondary outcomes included the proportion of patients prescribed an antibiotic on Day 0 and up to Day 14 at the health facility. Clinical outcomes included patient self-reported recovery at each follow-up visit; the duration and severity of symptoms; frequency of unplanned re-consultation within the 14 days of follow-up; temperature and CRP concentrations at Day 5 as objective measures of clinical recovery; and occurrence of serious adverse events (SAEs) as defined by requiring admission to hospital or death within 14 days of enrolment.

3.2.6 Retrospective baseline survey

We foresaw a potential for an observational bias due to the presence of research staff (the Hawthorne effect) and a possible contamination effect because health workers from the control group would also be exposed to the intervention (i.e. CRP test results following patient presentation), and may thus change their prescribing behaviours. Therefore, we carried out a baseline survey to capture prescribing practices in febrile patients prior to any intervention, relying on retrospective data.

In Thailand, a retrospective survey was carried out on routinely collected medical records from patients attending primary care facilities with a history of fever (within the last 14 days), documented temperature >37.5˚C, ICD-10 code for infection or those prescribed an antibiotic. Data were collected via a computerised search of patient records from the primary care facilities in the same area than the CRP Study, between January 2015 and December 2016. With the approval of the Chiang Rai Public Health Office (PHO), a research data manager accessed the PHO's routine medical records database to search for relevant patients and extract the pre-specified variables (history of fever, documented fever,

ICD-10 code for infection and antibiotic prescription). This survey will not be further described as it is part of another student's thesis, Rachel C. Greer, who coordinated the CRP Study in Thailand.

In Myanmar, data on antibiotics are routinely recorded in hard copy ("Drug book") by the facility staff based in the drug storage area in each site, controlling the drug delivery to the patient according to the healthcare worker prescription. This book is stored in a locked room outside of clinic hours. Monthly stock assessments ensure consistency between drug delivery and remaining stocks. Each patient's prescription including antibiotic type and dose was recorded in a "Drug book" records, however, the cause of the consultation (e.g. history of fever; febrile status; presence of an infection) was not recorded. Therefore, antibiotic prescription rate among febrile patients cannot be estimated.

Among the four Myanmar sites, only the government hospital outpatient department (OPD) had patient records that included clinical diagnosis including febrile status and antibiotic prescription, while in the three MAM clinics data were only available on the total number of non-routine visits (i.e. excluding those attending for HIV and TB care, antenatal clinic, family planning and malnourished children), without clinical diagnoses or febrile status, and only the overall number of antibiotics prescribed during the corresponding period was known. The baseline data collection was approved as a part of the study protocol by the Department of Medical Research Ethical Review Committee, and was carried out from November 2015 to April 2016.

3.2.7 Sample size calculation

Based on previous trials on CRP POCT, we anticipated CRP-testing to lower antibiotic prescriptions by 25% from baseline [116, 282]. However, we also expected contamination between study groups because health workers may modify their prescribing behaviour after being exposed to CRP POCT, so we increased the sample size with the intention to detect a reduction in antibiotic prescription rates of 20% independently for children and adults in Thailand and Myanmar. The sample size was also accounting for multiple comparisons between the three study groups [Group A 20 mg/L; Group B 40 mg/L and Group C, no CRP POCT] based on Bonferroni's correction. An adjusted significance level (type I error) of 0.017 was used to yield a 5% overall significance level for the three

comparisons. We anticipated a 15% loss to follow-up and finally aimed to recruit 198 patients per study group, rounded to 200, to reach a total of 2,400 patients (600 children and 600 adults per country).

3.2.8 Statistical analysis

We carried out an intention to treat analysis, as it allows for the provision of a pragmatic evaluation of CRP POCT. The *per-protocol* analysis was restricted to patients who complied with both Day 5 and Day 14 follow-up visits, and in whom healthcare workers strictly prescribed in accordance with the CRP results, which represents the potential impact of the test under full (and artificial) compliance.

Outcomes were measured overall and in the four pre-defined sub-groups of country and age category (adults defined as ≥12 years of age). We used means with standard deviations (SD) to describe continuous variables with normal distribution and medians with inter-quartile ranges (IQR) for continuous variables with a skewed distribution. Comparison between groups used t-tests for normally distributed variables, the Mann-Whitney test for non-normally distributed variables, and chi-squared test for categorical variables.

We also aimed to compare outcomes between each of the intervention groups and the controls with a logistic regression model; health facilities were considered to have a random effect on the primary outcome. Previous studies have indeed shown that prescribing behaviour is barely predictable, due to both health worker characteristics (age, time since graduation, personal preference) and patient expectations [35, 321-323]. Accounting for health centre as a random effect thus allowed to refine the interpretation of the CRP POCT impact on antibiotic prescription. In addition to the pre-designed statistical plan, we compared the impact of CRP POCT on broad-spectrum antibiotic prescriptions between the intervention groups and the controls, including ceftriaxone, cefixime, ciprofloxacin, levofloxacin, azithromycin and amoxicillin/clavulanic acid.

We analysed antibiotic reduction in regards to patient safety, and generated Kaplan-Meier curves to visualise time to clinical recovery based on the patient declaration, with a corresponding p-value using a Log-rank test. We also quantified the difference in clinical outcomes between intervention and control groups using a Cox regression model calculating the adjusted Hazard Ratios (HRs), with

age stratum and country as fixed effects and healthcare centres as a Gaussian random effect. The 95% confidence intervals have been provided where appropriate. Furthermore, we compared occurrence of SAEs, CRP levels and presence of fever at Day 5, as determined in the original statistical analysis plan.

Data were analysed with STATA version 15 (College Station, Texas, USA).

3.3 The CRP Study – Methods for causes of fever & performance of C-reactive protein in distinguishing bacterial from viral pathogens

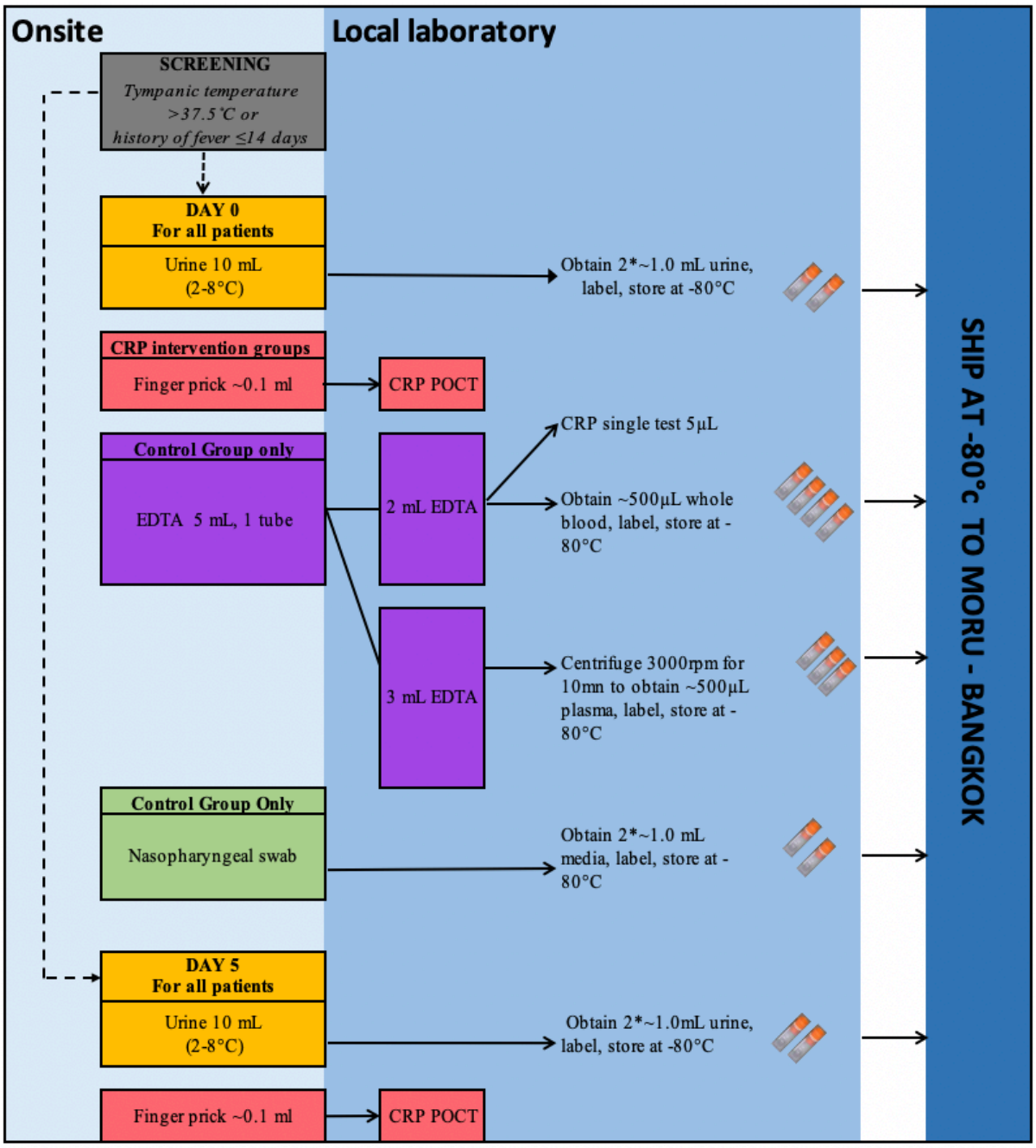
3.3.1 Clinical specimens

Children and adults randomised in the control group had blood specimens collected for off-site CRP testing (as compared with the intervention groups that had CRP tests performed on-site) and aetiological investigations.

Blood specimens were collected using ethylenediamine tetra-acetic acid (EDTA) tubes (Vacuette EDTA K3 tube, Greiner Bio-One International GmbH, Austria) in each primary care centre and transported in a cool box to a local laboratory. Plasma was obtained after centrifugation on the day of collection and was stored at -80˚C until analysis. A dried blood spot (DBS) was obtained from capillary blood specimens when venous blood was not collected, primarily in children under 5 in Myanmar. In addition to blood specimens, patients from the control group had a nasopharyngeal (NP) swab, using the Sigma VCM® (Wiltshire, England), which includes a cellular foam bud and a transport medium suitable for nucleic acid preservation and molecular amplification [324]. The study staff were trained for the swab procedure according to the WHO standards (https://www.who.int/influenza/rsv/rsv_collection_transport_storage_samples/en/). Swabs were then stored in a cool box after collection, and transported to a local laboratory on the same day for aliquoting and storage at -80˚C. Specimen processing is illustrated in Figure 3.1.

Microbiological investigations were carried out centrally at MORU in Bangkok, Thailand. We used a combination of molecular and serological assays to detect 35 bacteria, 30 viruses and three fungi from plasma and NP swab specimens as detailed below. Specimens and corresponding diagnostic methods are presented in Figure 3.2.

Figure 3-1 The CRP Study: specimen processing



Insufficient sample collection

Urine – if <1mL of urine collected, make 1 aliquot of 250/500/750µl of urine, depending on volume available

NP swab and viral transport media – sample likely to be present or absent due to patient intolerance

EDTA whole blood:

-If only 4mL of blood collected:

- 5µL for CRP POCT
- make **two** 500µL aliquots of whole blood
- centrifuge the remaining 3ml to obtain **three** 500µL aliquots of plasma

- If only 2mL of blood collected:

- 5µl for CRP POCT
- make **two** 500µL aliquots of whole blood
- centrifuge the remaining 1mL to obtain one 500µL aliquot of plasma

3.3.2 Taqman Array Card assay

3.3.2.1 Total nucleic acid preparation for Taqman Array Card assay

The total nucleic acid (TNA) was prepared from each 350 µl of plasma or NP swab using the MagNA Pure Compact Extractor (Roche Applied Sciences, Indianapolis, IN, USA), following manufacturer's instructions. For the pre-lysis step, Bacterial Lysis Buffer (BLB) (Roche, Germany) and proteinase K were added at the same specimen volume and 10% of the specimen volume, respectively.

3.3.2.2 Taqman Array Card assay procedure

The TaqMan Array Card (TAC) (Life Technologies, Foster City, USA) is a multiple-pathogen detection method based on real-time polymerase chain reaction (PCR) evaluated in detecting various respiratory, enteric pathogens in children and adults from both high-income countries and low-middle-income countries (LMICs) [325-328]. The TAC assay allows to customise the panel of pathogens targeted, with a minimal volume of specimen needed to test for up to 48 targets. Each card tests for six specimens at the same time with positive and negative controls in each card and within each patient ensure the quality of the PCR reaction, while ensuring a low risk of contamination due to the hermetic well plate format.

In this study, we customised the blood TAC assay to target nineteen bacteria, fourteen viruses and two parasites (Appendix VI). In addition, there were two extrinsic controls: one with the human RNase P-3 (RNP3) gene as an extraction and specimen integrity control, and another one as an internal positive control for the amplification (IPCO) [329-331]. For NP swabs, we customised the respiratory TAC assay to target sixteen viruses, sixteen bacteria and one fungus. Micro-organisms were selected in the panel according to those commonly found in Southeast Asia [6, 7, 15, 21, 107, 110].

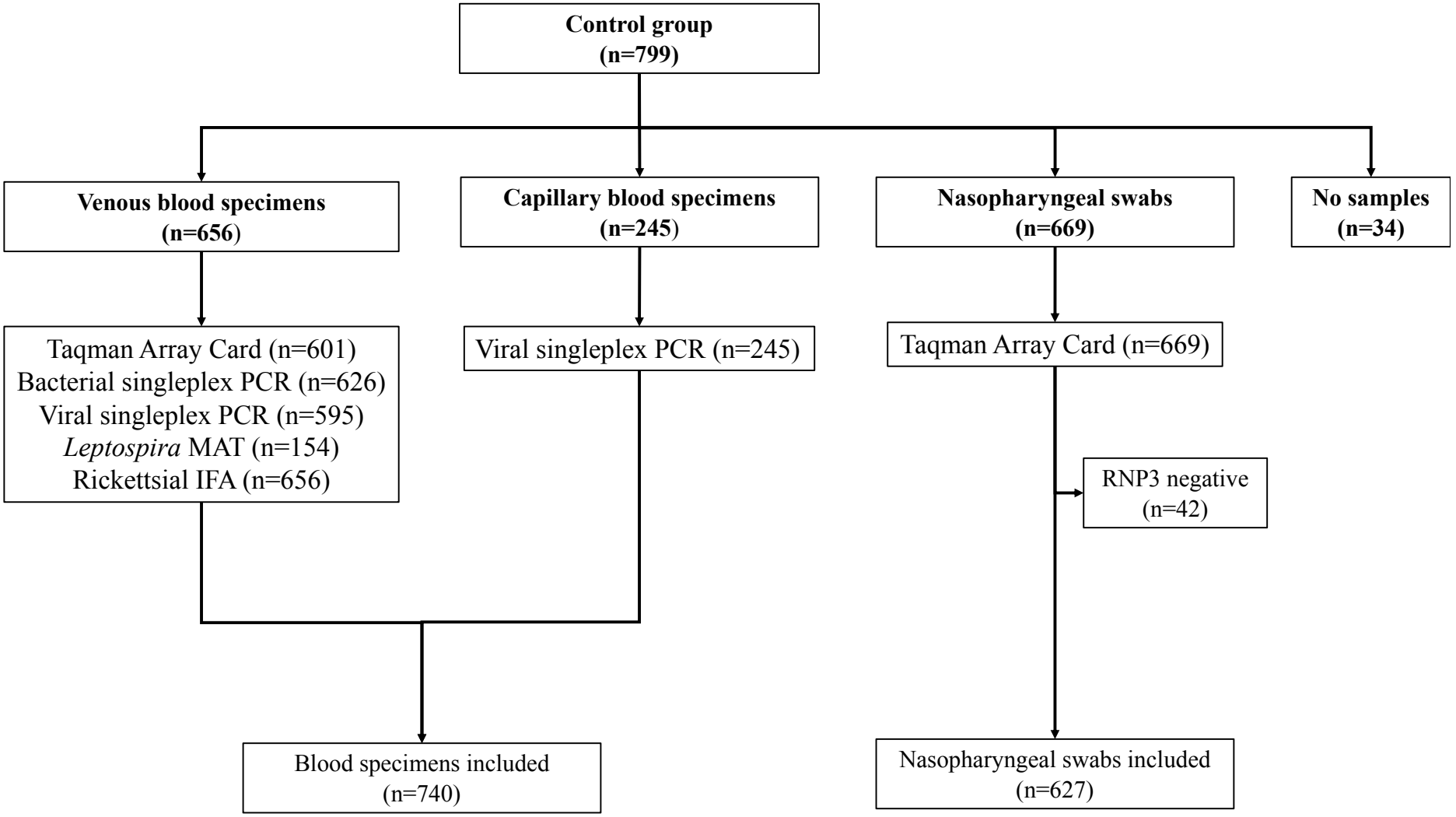


Figure 3-2 The CRP Study: specimen and diagnostic test

All assays were performed in duplicate to maximise sensitivity, and a coefficient of variation between the duplicates was calculated to ensure the TAC assay measurement consistency (Appendix VII). In addition to the six channels for patient's specimen, one channel with sterile water was used as a negative control ensuring the absence of contamination in the PCR, and one channel with positive control, with single-use aliquots of combined ribonucleic acid (RNA) transcript for positive amplification of all targets on all TAC formats, provided by the Centers for Diseases Control (CDC).

Each 50 µL of patient TNA, or negative control, or positive control was mixed with 50 µL of Quanta qScript XLT One-step reverse-transcriptase RT-qPCR ToughMix, low ROX (Quanta Biosciences, USA). The ToughMix enzyme mix contains all reagents, including the polymerase and reverse transcriptase enzymes, and has been demonstrated to enhance stability during PCR reactions [325]. Cards were centrifuged to homogeneously distribute the reaction mix to all wells (1 minute at 1,200 rpm twice) and sealed, following the manufacturer's instructions. Cards were run on the ViiA 7™ real-time PCR system (Life Technologies) using PCR cycling conditions comprising 10 min at 50˚C and 20 s at 95˚C followed by 45 two-step cycles of 3 s at 95˚C and 30 s at 60˚C as described previously [61].

3.3.3 Singleplex PCR assay

3.3.3.1 Singleplex PCR procedure

Dengue, chikungunya and zika viruses were further screened by a real-time RT-PCR assay, using the TaqMan® Fast Virus One-Step RT-PCR Master Mix (Applied Biosystems, Foster City, California) as described previously [332]. Performance of real-time RT-PCR on DBS for detecting these three arboviruses has been validated when compared with blood specimens [333].

Three probe-based real-time PCR assays further investigated *O. tsutsugamushi* (47 kDa *htrA* gene), *Rickettsia* spp. (17 kDa gene) as well as pathogenic *Leptospira* spp. targeting the *rrs* gene, as described previously [334-337]. We also screened for 16S RNA using an in-house real-time PCR targeting the 16S rRNA region V1 to V3 [338, 339]. For positive PCR, the products were undertaken for Sanger deoxyribonucleic acid (DNA) sequencing (Macrogen, Korea). We blasted the resulted 16S

rRNA sequences to National Center for Biotechnology Information (NCBI) data, operational taxonomic units (OTU) equal to or more than 97%.

3.3.3.2 DNA extraction for the singleplex PCR

Based on an in-house DNA extraction method (Gentra Puregene Blood kit, Qiagen, Norway), we used each 1 mL of whole blood for nucleic acid preparation. 3 mL of red blood cell (RBC) lysis solution was added to the whole blood and centrifuged at 3,000 rpm for two minutes. After discarding the supernatant, the remaining liquid was mixed with 1mL of cell lysis solution and 5 µL of RNase mixture. We added 333 µL protein precipitation solution (Gentra Puregene Blood kit, Qiagen, Norway), centrifuged at 3,000 rpm for 6 minutes and transferred the supernatant into a tube with undiluted isopropanol using a pasture pipette. We then centrifuged and discarded the supernatant, added 1 mL of 70% ethanol and inverted twice to wash the DNA pellet. Then, we centrifuged at 3,000 rpm for 1 minute at 25˚C, discarded the supernatant, centrifuged again at 3,000 rpm for 10 seconds and discarded all the solution using fine tip pasture pipette. We dried the pellet by leaving the tube open for 5 minutes, added a 100 µL preheated DNA Hydration Solution (Gentra Puregene Blood kit, Qiagen, Norway) at 65˚C, mixed and incubated at 65˚C for 1 hour.

3.3.4 Serology

Leptospirosis was also screened by IgM antibodies detection on a single acute specimen using a commercially available *Leptospira* IgM enzyme-linked immunosorbent assay (ELISA) (Panbio Pty., Ltd., Queensland, Australia). *Leptospira* IgM ELISA positive specimens were then confirmed by microscopic agglutination test (MAT), in microtiter plates and using reference strains of 24 *Leptospira interrogans* serovars [340]. The titre was defined as the final dilution that showed 50% agglutination. Reciprocal agglutination titres of greater than or equal to 50% were considered positive reactions. We regarded leptospiral MAT as positive for a titre ≥1:800 following the 2013 CDC recommendations (https://wwwn.cdc.gov/nndss/conditions/leptospirosis/case-definition/2013/).

Scrub typhus, typhus group and spotted fever group were also screened on a single acute specimen by IgM ELISA (InBios International Inc., Seattle, USA). Scrub typhus was confirmed by

indirect immunofluorescence assay (IFA) using slides coated with *O. tsutsugamushi* (strains Karp, Kato, and Gilliam) as previously described [58]. A stringent diagnostic positivity criterion was an admission IgM titre ≥1:3200 [169]. Typhus group and SFG were only considered probable cases using IFA by a single titre to ≥1:400, as no paired specimen was available [341].

3.3.5 Attribution of pathogenicity

A conservative diagnostic approach was chosen, following the strength of evidence for each result: any bacterium or virus detected in blood using the methods and thresholds detailed above was considered pathogenic. Considering NP swabs, only influenza virus (A and B), respiratory syncytial virus (RSV), human metapneumovirus and *Bordetella pertussis* were considered pathogenic. The corresponding evidence supporting pathogenicity in NP swabs is provided in Appendix VIII. In case of co-detection in blood and NP swabs, pathogens detected in blood were primarily considered.

Bacterial and viral aetiological groups included all cases where any bacteria or viruses were detected in blood, respectively, and only those organisms considered pathogenic in NP specimens.

3.3.6 Statistical analysis

We described organisms among children and adults (defined as ≥12 years of age) separately. Descriptive analysis for continuous variables with normal distribution used means and standard deviations (SD) and medians with inter-quartile ranges (IQR) for non-normally distributed continuous variables. Comparison between groups used t-tests for normally distributed variables, the Mann-Whitney test for non-normally distributed variables, and chi-squared test for categorical variables.

CRP values were compared across aetiological groups and clinical syndrome using the Mann-Whitney U test for two-group comparisons and the Kruskal-Wallis test for multi-group comparisons. Non-parametric receiver operating characteristic (ROC) curves were plotted and the Wald test was used to compare areas under the curve. Covariates included the following factors: patient age category and prior use of antibiotics. Diagnostic accuracy was assessed by calculating the areas under the ROC curves (AUC). An AUC of > 0.9 was considered excellent; 0.8-0.9, very good; 0.7-0.8, good; 0.6-0.7, average;

< 0.6, poor [342, 343]. Sensitivity, specificity, and percentage of correctly classified cases were also assessed for the two cut-off points used in the original trial: 20 mg/L and 40 mg/L and for current practice, based on observed prescribing practices at first presentation [100].

Data analyses were performed with STATA version 15 (College Station, Texas, USA).

3.4 The CRP Study – Methods for measuring the exposure to *Burkholderia pseudomallei*

Burkholderia pseudomallei is an environmental bacterium found in the tropics, and causes melioidosis. Approximately 165,000 cases are estimated globally every year, with about 40% of these in Southeast Asia where the mortality rate is 35% [344]. However, evidence on melioidosis is scarce in some Southeast Asian regions, and particularly in primary care, explaining why we decided to investigate human exposure to *B. pseudomallei* in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, using data from the CRP Study. A more detailed description of the rationale will be provided in the Chapter 6.

3.4.1 Clinical specimens

Of the 2,392 febrile children and adults recruited in the CRP Study between 2016-2017, 799 were randomly allocated to the control group (i.e. no C-reactive protein point-of-care test), in whom 656 had sufficient volume of blood for laboratory tests screening for haemolysin co-regulated protein 1 (Hcp1), as an indication of exposure to *Burkholderia pseudomallei*. In addition to these febrile patients, samples from 79 healthy controls (adults and children) living in Chiang Rai province were collected from 2015-2016. Of these, 41 (19 children and 22 adults) were matched by age category and subdistricts with two of the six original trial sites in Mueang Chiang Rai: Doi Hang and Mae Yao subdistricts.

All samples were stored at-80˚C until use, at MORU, Bangkok, Thailand.

3.4.2 Laboratory analysis

Plasma specimens were tested by ELISA using recombinant Hcp1, as previously described [345, 346].

3.4.3 Concept of exposure

Anti-Hcp1 and anti-OPS ELISA were only validated for attributing the diagnosis of acute infection to *B. pseudomallei*, and this, only among adults [231]. The antibody titre of blood culture-confirmed melioidosis cases was decreasing over 52 weeks in the *princeps* study of Pumpuang, suggesting a potential use for detecting recent exposure. However, no validation yet exists for ascertaining this suggestion. In the same way, no threshold has been validated to define seroprevalence. In our study, the measurement of anti-Hcp1 and anti-OPS ELISA can only be interpreted as a suspicion and not a demonstrated exposure to *B. pseudomallei*.

3.4.4 Statistical analysis

Differences in anti-Hcp1 IgG levels were analysed in pre-defined sub-groups of age category and country. Age category (adults defined as ≥18 years of age) was further analysed in patients under 7, 7-12, 13-18 and over 18 years old. Descriptive statistics for continuous variables with normal distribution used means and SD, and medians with IQR for non-normally distributed continuous variables. Comparison between groups used t-tests for normally distributed variables, the Mann-Whitney test for non-normally distributed variables, and chi-square test for categorical variables. Pearson's correlation was calculated between anti-Hcp1 and age, as well as between anti-Hcp1 and OPS IgG levels, including p-values.

Statistical analysis was carried out using STATA version 15 (College Station, Texas, USA). The medians of anti-Hcp1 IgG levels of patients for all Mueang Chiang Rai sites were mapped using the ggplot2 package of R language [347, 348].

3.5 The CRP Study – Ethical approval

The protocol, informed consent form and case record forms were reviewed and approved by the Oxford Tropical Research Ethics Committee (OxTREC, reference 49-15), the Mahidol University Faculty of Tropical Medicine Ethics Committee (FTMEC, reference TMEC 16-015), and the Myanmar Department of Medical Research (DMR, reference Ethics/DMR/2016/137) and Chiang Rai Provincial Public Health Office Research Ethics Committees (CR PHO EC, reference MO/002.17/233).

This study was supported by a Wellcome Trust/ISSF grant (105605/Z/14/Z) and with the support of the Foundation for Innovative New Diagnostics (FIND) funding from the Australian government.

3.6 The Tanzanian Study – Methods

3.6.1 Clinical specimens

This study was carried out in two sentinel hospitals in Moshi, northern Tanzania: the medical ward of Kilimanjaro Christian Medical Centre, and the medical ward, paediatric ward, and outpatient department of Mawenzi Regional Referral Hospital.

Hospitalised patients from the medical ward were eligible if they had either a history of fever of less than three days, or a documented fever (axillary temperature >37.5˚C or a tympanic, oral or rectal temperature ≥38˚C during screening). Outpatients from the outpatient department were only eligible in case of a documented fever. An informed consent was collected for each patient, and parental consent in the case of patients under 18 years of age. Demographic and physical examination data were collected by a study clinician on a case report form (CRF), as well as clinical outcome as discharged, transferred, or dead. We defined children as patients under 10 years of age, following WHO standards [349].

3.6.2 Laboratory analysis

Blood was prospectively sampled from patients with an acute fever, and details of the laboratory analysis have been reported [134].

Pathogens were classified in different aetiological groups consisting of either bacterial infections, fungal blood stream infection (BSI), malaria, or undetermined. Bacterial infections were also considered by the mode of diagnostic method, with BSI diagnosed by blood culture and bacterial zoonosis by serology. Among patients with co-infections from different aetiological groups, those with a bacterial BSI were considered bacterial BSI. Patients with a bacterial zoonotic pathogen and malaria co-infection were considered bacterial zoonosis.

For patients with sufficient sample volumes available, CRP levels were measured at the Intermountain Central Laboratory (Murray, USA), using the Multigent Vario assay on the Abbott c8000

chemistry analyser (Abbott, Abbott Park, USA). None of these assessments were communicated to the clinicians.

3.6.3 Statistical analysis

Differences in median CRP levels were measured using the Wilcoxon rank-sum test. We estimated the sensitivity to identify bacterial infections and the corresponding confidence intervals using the Wilson score method. We also aimed to report the CRP levels and performance among patients with fungal BSI and malaria, as well as the CRP distribution across inpatients and outpatients and to compare antibacterial prescription between standard-of-care practice and CRP-guided prescription estimates, including broad-spectrum antibacterials. We considered ceftriaxone, ciprofloxacin, azithromycin, and amoxicillin/clavulanic acid broad-spectrum antibacterials [350, 351].

3.6.4 Ethical approval

The Kilimanjaro Christian Medical University College Research Ethics Committee (approval #295) approved this study, the Tanzania National Institute for Medical Research National Ethics Coordinating Committee (approval NIMR/HQ/R.8a/Vol.IX/1000), and an Institutional Review Board of the Duke University Hospital System (approval IRB#Pro00016134).

Chapter 4

THE CRP STUDY – IMPACT OF C-REACTIVE PROTEIN ON ANTIBIOTIC PRESCRIPTION IN PRIMARY CARE

4 THE CRP STUDY – IMPACT OF C-REACTIVE PROTEIN ON ANTIBIOTIC PRESCRIPTION IN PRIMARY CARE

4.1 Research in context

In Southeast Asia, a single study found a modest but significant antibiotic reduction from 78 to 64% using C-reactive protein-testing at point-of-care (CRP POCT) in Vietnam in 2016, but only among primary care patients with respiratory symptoms, and testing a single CRP threshold per age category (10 mg/L in children and 20 mg/L in adults) [274]. Considering low-to-middle-income countries (LMICs) as a whole, another study based in Tanzania largely reduced antibiotic prescription among children with acute respiratory infection (from 94.9% to 11.5%), but CRP was tested within a strict electronic algorithm and combined with additional diagnostic tools (malaria rapid diagnostic test, haemoglobin, oximeter, procalcitonin and a glucometer) [99]. All these studies considered antibiotic prescription at study sites the primary outcome, bearing in mind that patients may bypass the prescriber's decision and obtain an antibiotic elsewhere, particularly from pharmacies [171].

We aimed to extend CRP POCT evidence to all febrile children and adults regardless of their clinical presentation, and to prospectively compare two different CRP thresholds (20 and 40 mg/L) on antibiotic prescription. We will here only present findings from the Myanmar side of the CRP Study, based in three primary care clinics and one outpatient department from a public hospital in Hlaing Tha Yar, in the west of Yangon. The Thai side of this study will be included in another doctoral Thesis, Dr Rachel C. Greer, who co-ordinated activities in the Thai sites and was a co-author on the paper published describing the main trial (Appendix IX).

4.2 Results

4.2.1 Baseline survey

In order to capture routine prescribing behaviours, we retrospectively reviewed antibiotic prescription in three Medical Action Myanmar (MAM) clinics where we aimed to implement the CRP Study, as well as in one outpatient department of a governmental hospital, all based in Hlaing Tha Yar, Myanmar. In the hospital, we accessed patient's febrile status in addition to antibiotic prescription, including 280 febrile outpatients between November 2015 and April 2016. The corresponding prescription proportion was 68.6%. In the three MAM clinics, we gathered 32,065 non-routine patient's data over the same period of time, but the febrile status nor the cause of attendance were not recorded. Among all these patients (both febrile and afebrile), 13,206 (41.1%) of them had an antibiotic prescribed (Figure 4.1).

4.2.2 Patients characteristics

Among the 1,228 patients prospectively recruited with a documented fever (tympanic temperature >37.5˚C) or a history of fever (less than 14 days) between November 2016 and August 2017, 619 were children (under 12 years of age) and 609 were adults (Figure 4.2). Demographic and clinical characteristics are presented in Table 4.1.

Patients from the control group and the two CRP intervention groups combined were similar: patients attended primary care 2-3 days after symptom onset (interquartile range [IQR] 2-5). Clinically, more than half of patients had a documented fever, and more than two-third presented with respiratory symptoms. The second most frequent presentation was gastro-intestinal symptoms, followed by neurological and other symptoms.

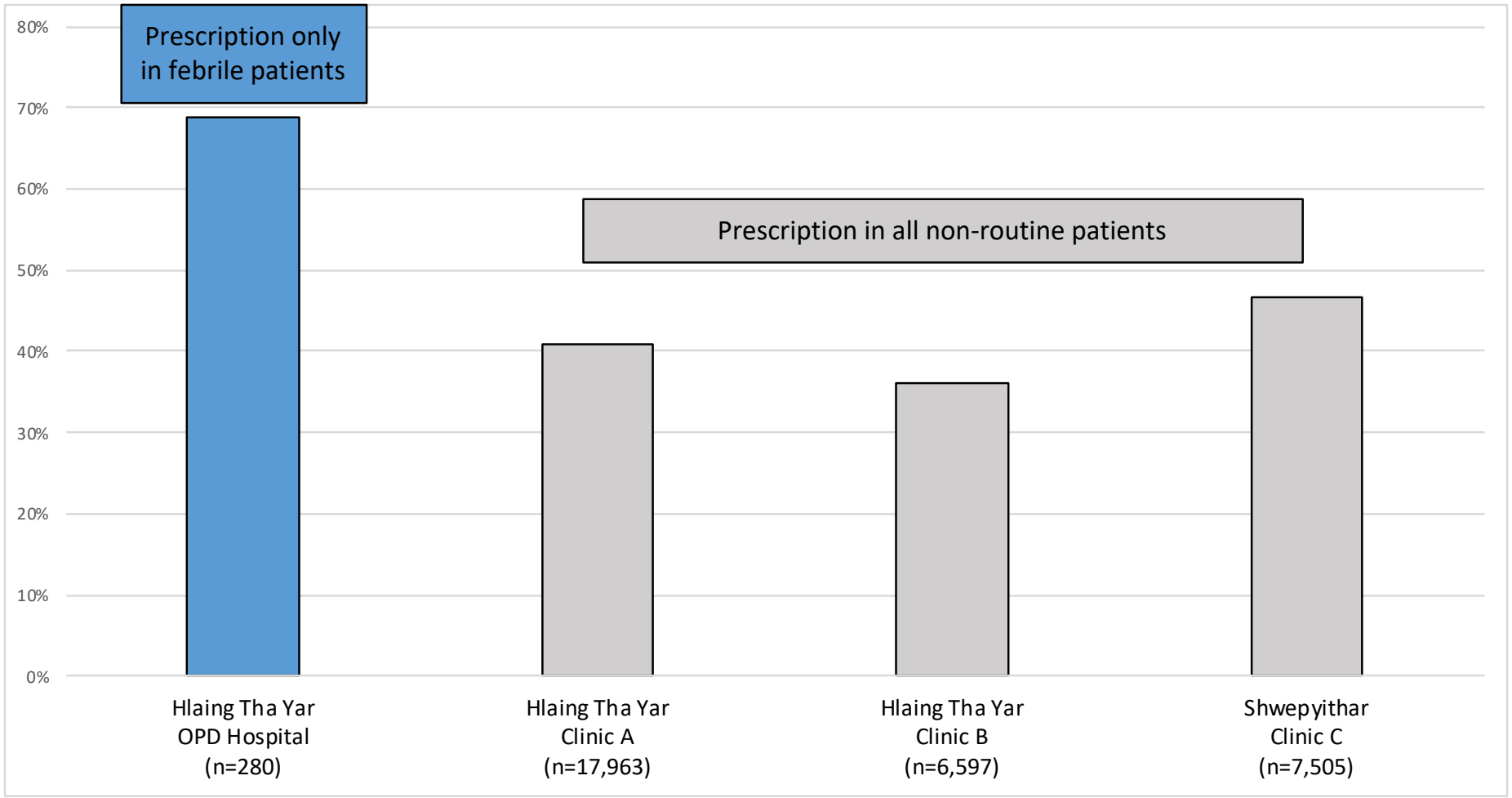


Figure 4-1 Baseline antibiotic prescribing in Hlaing Tha Yar, Lower Myanmar, 2015-2016.

Bar in blue indicates estimates for which clinical data on febrile status were available, while those in grey convey the estimated prescription in all non-routine visits.

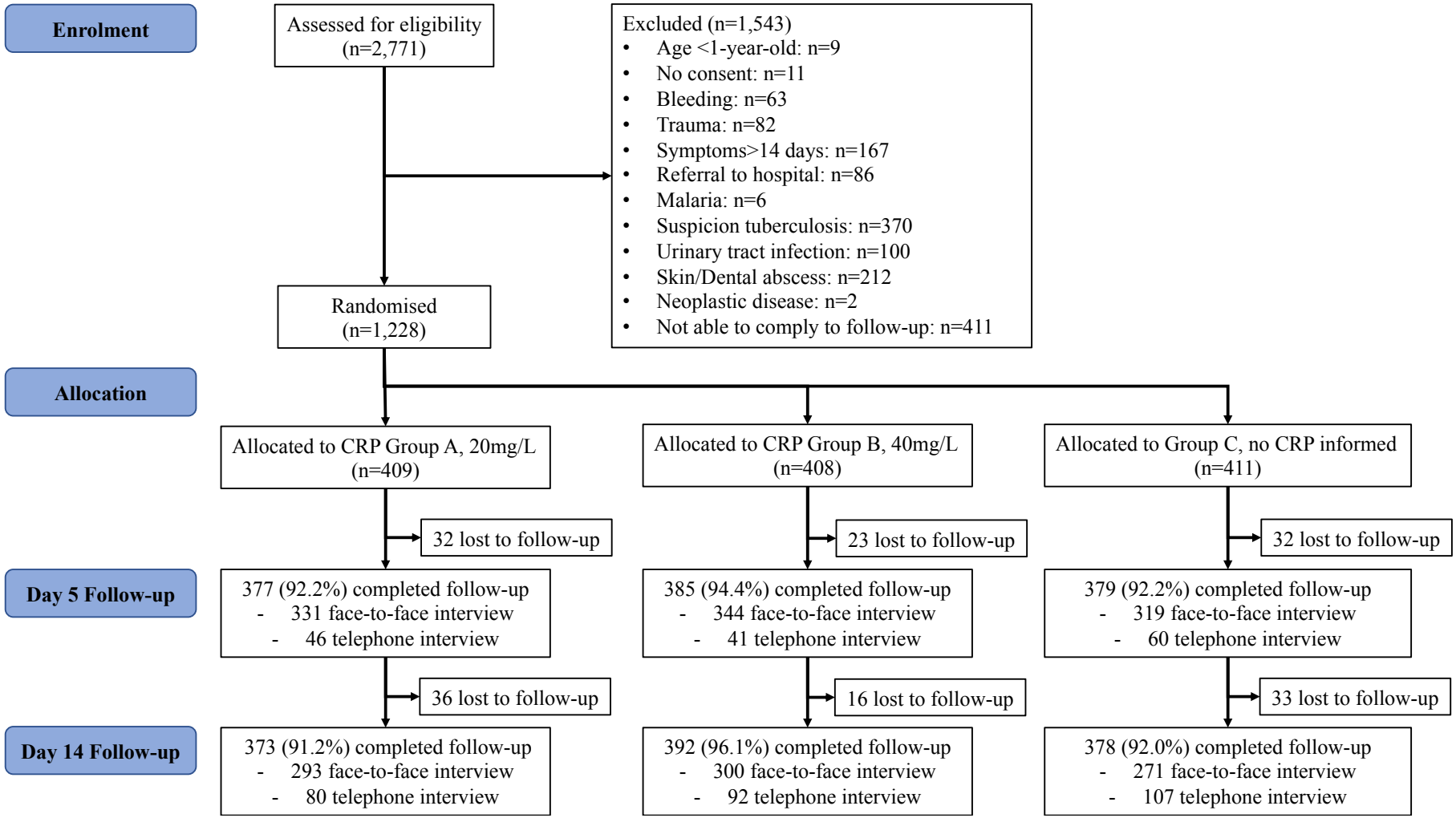


Figure 4-2 The CRP Study: trial flow chart in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Table 4-1 Day 0 characteristics comparing controls and combined CRP groups per age category (adults defined as ≥12 years of age) in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Day 0 characteristics	Children (n=619)		Adults (n=609)	
	Controls (n=207)	CRP groups (n=412)	Controls (n=204)	CRP groups (n=405)
Demographic characteristics				
Male, n (%)	103 (49.8)	207 (50.2)	76 (37.3)	168 (41.5)
Age, median (IQR)	3 (2-6)	4 (2-6)	29 (19-42)	28 (20-43)
Presence of comorbidity, n (%)	7 (3.4)	14 (3.4)	63 (30.9)	96 (23.7)
Symptom onset (in days), median (IQR)	2 (2-3)	3 (2-3)	3 (2-5)	3 (2-5)
≥ 30min to reach the facility, n (%)	55 (26.6)	120 (29.1)	50 (24.5)	121 (29.9)
Self-reported antibiotic intake, n (%)	7 (3.4)	26 (6.3)	7 (3.4)	21 (5.2)
Clinical characteristics				
Documented fever (>37.5°C), n (%)	131 (63.3)	279 (67.7)	104 (51.0)	183 (45.2)
Neurological symptoms, n (%)	11 (5.3)	19 (4.6)	63 (30.9)	133 (32.8)
Respiratory symptoms, n (%)	151 (73.0)	299 (72.6)	145 (71.1)	263 (64.9)
Gastrointestinal symptoms, n (%)	63 (30.4)	144 (35.0)	49 (24.1)	95 (23.5)
Other symptoms, n (%)	26 (12.6)	43 (10.4)	15 (7.4)	62 (15.3)

Comorbidities included HIV, chronic hepatitis B or C, cirrhosis, diabetes mellitus, asthma, anaemia, chronic obstructive pulmonary disease, gastritis, congenital heart or kidney disease, alcoholism, dyslipidaemia, glucose-6-phosphate dehydrogenase (G6PD) deficiency, hypertension, rheumatic heart disease, thalassaemia, thyroid disease.
Neurological symptoms include headache, confusion, dizziness or hearing loss.
Respiratory symptoms include sore throat, dyspnoea, chest pain, runny nose, or cough.
Gastro-intestinal symptoms include nausea, vomiting, diarrhoea, or abdominal pain.
Other symptoms declared were defined by the presence of fever alone or symptoms other than those present in neurological, respiratory, or gastrointestinal symptoms. Common symptoms in this group included myalgia, arthralgia, jaundice, tiredness, chills, sweating, weight loss, skin eruption, dysuria, or eye redness.

4.2.3 Antibiotic prescribing

The prescription level in the controls was 42.2 (170/403), 47.6 (186/391) and 48.7% (190/390) at Day 0, between Day 0 and Day 5, and between Day 0 until Day 14, respectively.

Considering our primary endpoint, antibiotic prescription was significantly reduced between Day 0 and Day 5 when comparing the controls with Group B, as shown in Table 4.2. The reduction was of 8.6 percentage-points (risk difference [RD] -8.6, 95% confidence interval [-15.5, -1.7], adjusted odds ratio [aOR] OR 0.68 [0.51, 0.91]), and was of a greater magnitude among adults (-11.9 [-21.7, -2.2], aOR 0.59 [0.39, 0.88]) but not significant among children (-5.3 [-15.0, 4.4], aOR 0.80 [0.53, 1.20]). Antibiotic prescription remained significantly reduced considering the whole follow-up period from Day 0 until Day 14 (RD -7.8 [-14.6, -1.0], aOR 0.67 [0.50, 0.89]). The greatest reduction was observed both at enrolment and during the whole follow-up period among adults, with a reduction of 13.3 percentage-points, while the reduction on this period was non-significant among children (RD -4.3 [-14.1, 5.4], aOR 0.83 [0.55, 1.25]). In Group A, antibiotic prescription was non-significantly lowered between Day 0 and Day 5 (RD -4.2 [-11.2, 2.7], aOR 0.84 [0.63, 1.12]), nor at any time points of the follow-up.

Table 4-2 Antibiotic prescription in the controls, Group A (20 mg/L) and Group B (40 mg/L) in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Controls	Group A	RD (95%CI)	aOR* (95% CI)	Group B	RD (95%CI)	aOR* (95% CI)
Overall							
On Day 0, n (%)	170/403 (42.2)	156/403 (38.7)	-3.5 (-10.3, 3.3)	0.87 (0.66, 1.16)	131/404 (32.4)	-9.8 (-16.4, -3.1)	0.67 (0.50, 0.89)
Between Day 0 – Day 5, n (%)	**186/391 (47.6)**	**169/390 (43.3)**	**-4.2 (-11.2, 2.7)**	**0.84 (0.63, 1.12)**	**155/398 (38.9)**	**-8.6 (-15.5, -1.7)**	**0.68 (0.51, 0.91)**
Between Day 0 – Day 14, n (%)	190/390 (48.7)	171/383 (44.7)	-4.1 (-11.1, 3.0)	0.85 (0.64, 1.13)	158/396 (39.9)	-8.8 (-15.7, -1.9)	0.68 (0.51, 0.90)
Children							
On Day 0, n (%)	78/203 (38.4)	77/204 (37.8)	-0.7 (-10.1, 8.8)	0.99 (0.66, 1.48)	65/204 (31.9)	-6.6 (-15.8, 2.7)	0.76 (0.50, 1.15)
Between Day 0 – Day 5, n (%)	**87/195 (44.6)**	**84/195 (43.1)**	**-1.5 (-11.4, 8.3)**	**0.95 (0.63, 1.42)**	**79/201 (39.3)**	**-5.3 (-15.0, 4.4)**	**0.80 (0.53, 1.20)**
Between Day 0 – Day 14, n (%)	88/195 (45.1)	86/189 (45.5)	0.4 (-9.6, 10.3)	1.03 (0.69, 1.55)	82/201 (40.8)	-4.3 (-14.1, 5.4)	0.83 (0.55, 1.25)
Adults							
On Day 0, n (%)	92/200 (46.0)	79/199 (39.7)	-6.3 (-16.0, 3.4)	0.78 (0.52, 1.15)	66/200 (33.0)	-13.0 (-22.5, -3.5)	0.58 (0.38, 0.87)
Between Day 0 – Day 5, n (%)	**99/196 (50.5)**	**85/195 (43.6)**	**-6.9 (-16.8, 3.0)**	**0.75 (0.50, 1.12)**	**76/197 (38.6)**	**-11.9 (-21.7, -2.2)**	**0.59 (0.39, 0.88)**
Between Day 0 – Day 14, n (%)	102/195 (52.3)	85/194 (43.8)	-8.5 (-18.4, 1.4)	0.71 (0.47, 1.06)	76/195 (39.0)	-13.3 (-23.1, -3.5)	0.55 (0.37, 0.84)

The primary trial outcome (i.e. the prescription of antibiotics from Day 0 up to Day 5) is in **bold**
RD: risk difference
95% CI: 95% confidence interval
aOR: adjusted odds ratio

Considering the *per-protocol* analysis, 968 patients were included (78.8% of the intention-to-treat [ITT] analysis), including 486 children and 482 adults. As a reminder, the *per-protocol* analysis was limited to patients who strictly complied to each follow-up visit and for whom health workers systematically complied with the CRP results. We found a greater reduction in the primary outcome compared with the ITT analysis, with a 21 percentage-point reduction (RD -21.0 [-27.9, -14.2], aOR 0.37 [0.27, 0.53]), and this significant difference was also observed among children and adults separately (Table 4.3). The greatest reduction was also observed among adults between Day 0 and Day 14 of the follow-up, by over 25 percentage-points (RD -25.3 [35.2, -15.3], aOR 0.32 [0.19, 0.49]).

Table 4-3 Antibiotic prescription in the controls, Group A (20 mg/L) and Group B (40 mg/L) in Hlaing Tha Yar, Lower Myanmar, 2016-2017, using a *per-protocol* analysis.

	Controls	**Group A**	**RD (95%CI)**	**aOR* (95% CI)**	**Group B**	**RD (95%CI)**	**aOR* (95% CI)**
Overall	*n=374*	*n=291*			*n=303*		
On Day 0, n (%)	158 (42.3)	96 (33.0)	-9.3 (-16.6, -1.9)	0.68 (0.49, 0.94)	62 (20.5)	-21.8 (-28.5, -15.0)	0.35 (0.25, 0.50)
Between Day 0 - Day 5, n (%)	**160 (42.8)**	**98 (33.7)**	**-9.1 (-16.5, -1.7)**	**0.68 (0.50, 0.91)**	**66 (21.8)**	**-21.0 (-27.9, -14.2)**	**0.37 (0.27, 0.53)**
Between Day 0 - Day 14, n (%)	164 (43.9)	100 (34.4)	-9.5 (-16.9, -2.1)	0.67 (0.49, 0.93)	69 (22.8)	-21.1 (-28.0, -14.2)	0.38 (0.27, 0.53)
Children	*n=185*	*n=145*			*n=156*		
On Day 0, n (%)	71 (38.4)	51 (35.2)	-3.2 (-13.7, 7.3)	0.89 (0.57, 1.41)	33 (21.2)	-17.2 (-26.7, -7.7)	0.44 (0.27, 0.72)
Between Day 0 - Day 5, n (%)	**77 (41.6)**	**57 (39.3)**	**-2.3 (-13.0, 8.4)**	**0.91 (0.58, 1.42)**	**46 (29.5)**	**-12.1 (-22.2, -2.1)**	**0.60 (0.38, 0.94)**
Between Day 0 - Day 14, n (%)	78 (42.2)	59 (40.7)	-1.5 (-12.2, 9.2)	0.94 (0.61, 1.46)	49 (31.4)	-10.8 (-20.9, -0.6)	0.64 (0.41, 1.00)
Adults	*n=189*	*n=146*			*n=147*		
On Day 0, n (%)	87 (46.0)	45 (30.8)	-15.2 (-25.5, -4.9)	0.52 (0.33, 0.82)	29 (19.7)	-26.3 (-35.9, -16.7)	0.28 (0.17, 0.47)
Between Day 0 - Day 5, n (%)	**94 (49.7)**	**50 (34.3)**	**-15.5 (-26.0, -5.0)**	**0.52 (0.34, 0.82)**	**36 (24.5)**	**-25.3 (-35.2, -15.3)**	**0.32 (0.20, 0.52)**
Between Day 0 - Day 14, n (%)	97 (51.3)	50 (34.3)	-17.1 (-27.6, -6.6)	0.49 (0.31, 0.77)	36 (24.5)	-26.8 (-36.8, -16.9)	0.30 (0.19, 0.49)

The primary trial outcome (i.e. the prescription of antibiotics from Day 0 to Day 5) is in **bold**
RD: risk difference
95% CI: 95% confidence interval
aOR: adjusted odds ratio

The trial primary outcome also evaluated antibiotic prescription in relation to the CRP thresholds, as shown in Figure 4.3. Patients with high CRP levels, above 20 or 40 mg/L, had higher antibiotic prescription in the intervention groups compared with the controls (p-value <0.001). Correspondingly, patients with low CRP levels, below 20 or 40 mg/L had lower antibiotic prescription in the intervention groups than in the controls (p-value <0.001).

Assuming a threshold of 20 mg/L as indicative of the need of antibiotic, 58.1% (154/265) of the controls had an antibiotic correctly prescribed/withheld, as compared with 78.9% (321/407) in Group A. Similarly, 58.9% (156/265) of controls had an antibiotic appropriately prescribed/withheld, as compared with 79.4% (314/408) in Group B, using a 40 mg/L threshold as indicative of requiring an antibiotic. During the trial, primary health workers followed the CRP test results by almost 80%, both in Groups A and B.

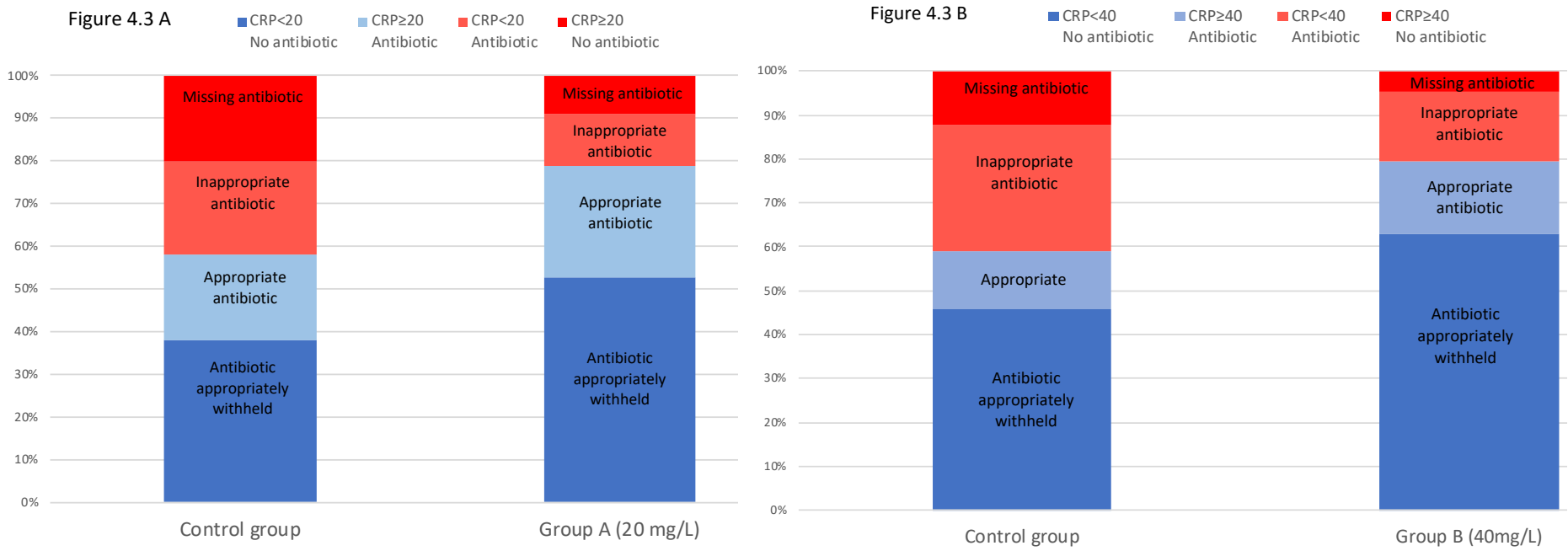


Figure 4-3 Antibiotic prescribing on Day 0 in relation to the CRP thresholds in Groups A (20 mg/L) and B (40 mg/L) for all age categories in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Figure 4.3 A shows antibiotic prescription for a 20 mg/L threshold; Figure 4.3 B for a 40 mg/L threshold.

When considering patients with a documented fever, there was a greater reduction of the trial primary outcome than all patients with either a documented fever or a history of fever, with a reduction of 11.1 percentage-points between Day 0 and Day 5 in Group B compared with the controls (RD -11.1 [-20.2, -1.9], aOR 0.59 [0.41, 0.87]), and this difference remained significant at all time points of the follow-up (Table 4.4).

The risk-difference was also significant in the subgroup of patients with either a neurological or a respiratory presentation for the primary endpoint, but not among those with gastro-intestinal symptoms. Respiratory and neurological presentations were the syndromes with the greatest antibiotic reduction, found at all points of the follow-up except at Day 0 for neurological symptoms (RD -12.5 [-27.7, 2.8], aOR 0.50 [0.24, 1.04]).

In Group A, antibiotic reduction was not significant in any of the clinical sub-groups, and there was a non-significant increase when considering patients with a gastro-intestinal presentation (RD 2.1 [-10.8, 15.0], aOR 1.08 [0.63, 1.85]).

Table 4-4 Subgroup analysis for antibiotic prescription in the controls, Group A (20 mg/L) and Group B (40 mg/L) in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Controls	Group A	RD (95%CI)	aOR* (95% CI)	Group B	RD (95%CI)	aOR* (95% CI)
Neurological presentation							
On Day 0, n (%)	32/74 (43.2)	31/74 (41.9)	-1.4 (-17.3, 14.6)	0.91 (0.47, 1.77)	24/78 (30.8)	-12.5 (-27.7, 2.8)	0.50 (0.24,1.04)
Between Day 0 - Day 5, n (%)	**36/71 (49.3)**	**34/71 (47.9)**	**-1.4 (-17.8, 14.9)**	**0.94 (0.48, 1.81)**	**23/76 (30.3)**	**-19.1 (034.5, -3.6)**	**0.36 (0.17, 0.77)**
Between Day 0 - Day 14, n (%)	37/73 (50.7)	34/71 (47.9)	-2.8 (-19.1, 13.5)	0.89 (0.46, 1.72)	23/75 (30.7)	-20.0 (-35.5, -4.5)	0.35 (0.17, 0.74)
Respiratory presentation							
On Day 0, n (%)	144/296 (48.7)	117/281 (41.6)	-7.0 (-15.1, 1.1)	0.77 (0.55, 1.08)	95/281 (33.8)	-14.8 (-22.8, -6.9)	0.52 (0.37, 0.74)
Between Day 0 - Day 5, n (%)	**157/286 (54.9)**	**128/270 (47.4)**	**-7.5 (-15.8, 0.8)**	**0.76 (0.54, 1.07)**	**118/274 (43.1)**	**-11.8 (-20.1, -3.6)**	**0.60 (0.43, 0.85)**
Between Day 0 - Day 14, n (%)	159/286 (55.6)	129/268 (48.1)	-7.5 (-15.8, 0.8)	0.76 (0.54, 1.07)	121/273 (44.3)	-11.3 (-19.5, -3.0)	0.62 (0.44, 0.87)
Gastro-intestinal presentation							
On Day 0, n (%)	38/112 (33.9)	47/125 (37.6)	3.7 (-8.5, 15.9)	1.17 (0.69, 2.00)	36/114 (31.6)	-2.3 (-14.6, 9.9)	0.86 (0.49, 1.52)
Between Day 0 - Day 5, n (%)	**44/107 (41.1)**	**51/118 (43.2)**	**2.1 (-10.8, 15.0)**	**1.08 (0.63, 1.85)**	**39/112 (34.8)**	**-6.3 (-19.1, 6.5)**	**0.71 (0.40, 1.25)**
Between Day 0 - Day 14, n (%)	44/106 (41.5)	52/115 (45.2)	3.7 (-9.4, 16.8)	1.16 (0.68, 1.98)	39/111 (35.1)	-6.4 (-19.3, 6.5)	0.71 (0.40, 1.25)
Documented fever							
On Day 0, n (%)	110/235 (46.8)	103/231 (44.6)	-2.2 (-11.3, 6.8)	0.91 (0.63, 1.31)	81/231 (35.1)	-11.7 (-20.6, -2.9)	0.58 (0.40, 0.85)
Between Day 0 - Day 5, n (%)	**117/226 (51.8)**	**109/220 (49.6)**	**-2.2 (-11.5, 7.1)**	**0.90 (0.62, 1.31)**	**92/226 (40.7)**	**-11.1 (-20.2, -1.9)**	**0.59 (0.41, 0.87)**
Between Day 0 - Day 14, n (%)	118/226 (52.2)	110/216 (50.9)	-1.3 (-10.6, 8.0)	0.94 (0.64, 1.37)	93/225 (41.3)	-10.9 (-20.0, -1.7)	0.60 (0.41, 0.88)

The primary trial outcome (i.e. the prescription of antibiotics from Day 0 up to Day 5) is in **bold**
RD: risk difference
95% CI: 95% confidence interval
aOR: adjusted odds ratio

Antibiotics prescribed at the facilities included β-lactam molecules (penicillin V, penicillin G, ampicillin, amoxicillin, cloxacillin, dicloxacillin, amoxicillin/clavulanic acid, cefalexin, cefixime and ceftriaxone), macrolides (erythromycin, roxithromycin and azithromycin), quinolones (norfloxacin, levofloxacin, ciprofloxacin), and tetracycline (doxycycline), as well as metronidazole and co-trimoxazole (trimethoprim/sulfamethoxazole). From these, broad-spectrum antibiotics included amoxicillin/clavulanic acid, cefixime, ceftriaxone, azithromycin, levofloxacin and ciprofloxacin.

Considering broad-spectrum antibiotics, the prescription was significantly reduced in Group B compared with the controls at Day 0, between Day 0 and Day 5, and between Day 0 until Day 14 of the follow-up (aOR 0.50 [0.30, 0.84], aOR 0.66 [0.50, 0.89], and aOR 0.67, [0.50, 0.89], respectively). This reduction was not significant in Group A compared with the controls (data not shown).

The commonest antibiotic prescribed was amoxicillin with 59.8% (101/169) and 65.6 (189/288) of the prescriptions at Day 0 in the controls and the combined CRP groups, respectively. Other antibiotics were used in less than 15% of the cases, with cephalosporin and tetracycline/macrolide being the second and third most frequently prescribed antibiotics (14.8% [25/169] in the controls, 12.5% [36/288] in the CRP combined groups, and (11.2% [19/169] in the controls, 8.3% [24/288] in the CRP combined groups, respectively). The least prescribed molecules were cotrimoxazole and metronidazole, in less than 3% of the cases (Figure 4.4).

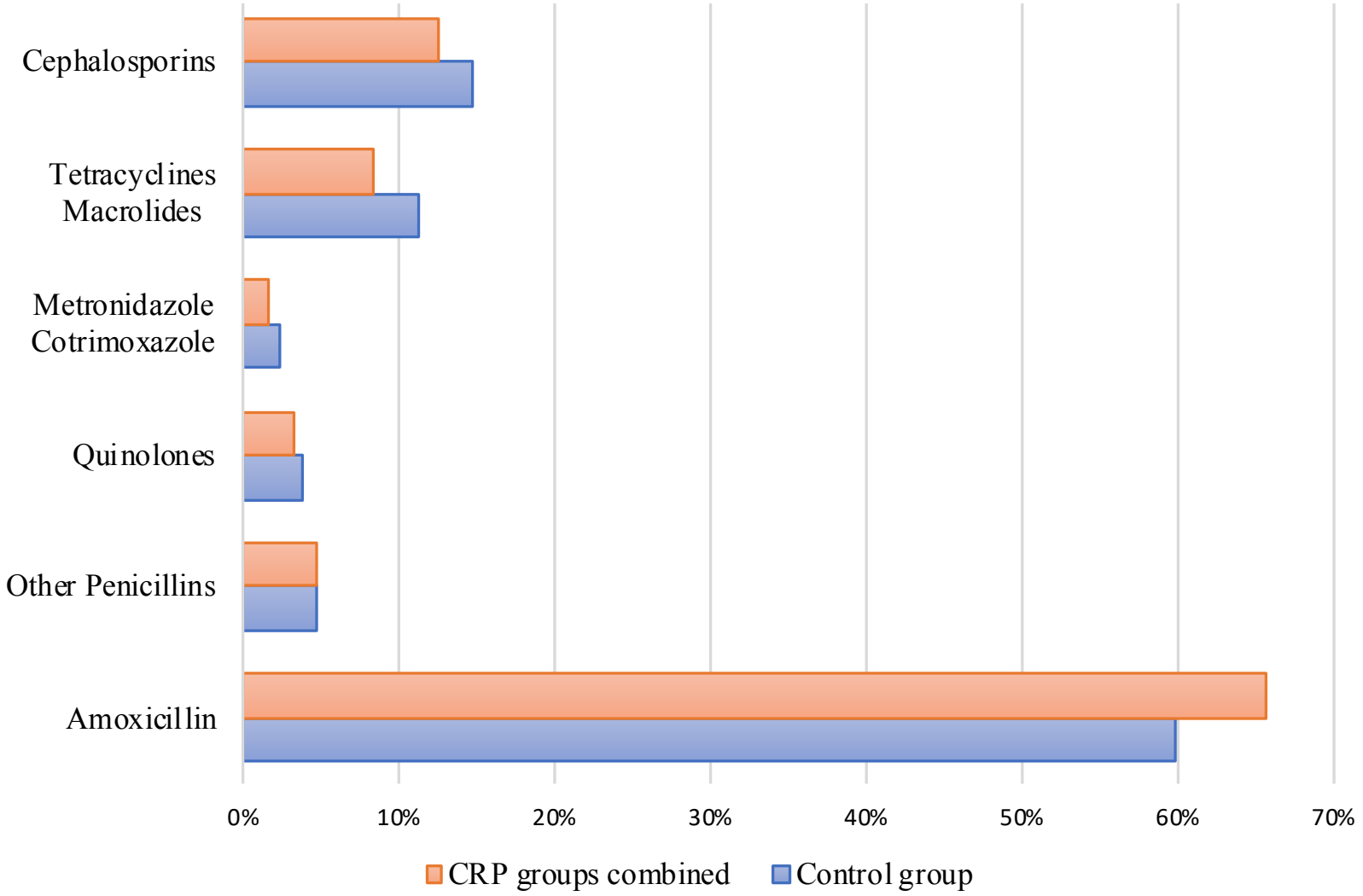


Figure 4-4 Antibiotic prescription in the controls comparing to the CRP combined groups (Groups A and B) on Day 0 in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

4.2.4 Clinical outcomes

All endpoints used to measure clinical recovery were similar between the controls, Group A and Group B: according to patient's declaration, illness was persisting at Day 5 in 28.9% (108/374), 22.8% (85/373) and 27.4% (105/383), respectively (Table 4.5). Clinical recovery remained similar between groups until Day 14 of the follow-up, measured at 4.7% (19/403), 5.7% (23/403) and 6.2% (25/404). Other endpoints included symptom severity, the presence of a documented fever and frequency of unscheduled visits, and were also similar between the three groups during the whole follow-up period. The proportion of patients with elevated CRP at Day 5 (≥50 mg/L in children and ≥100 mg/L in adults) was not significantly different between the controls (23.8% [96/403]) and Group A (19.9% [80/403], p-value ≥0.05) but significantly higher than in Group B (15.8% [64/404], p-value 0.004).

Recovery comparison was illustrated using survival curves (Figure 4.5), and corresponding Log-rank test between the controls and Group A (p-value 0.565) and Group B (p-value 0.773) were not significant. Hazard ratios (HR) quantifying recovery comparing the controls with Groups A and B did not show any significant difference either: HR 1.04 (0.90-1.21) and 1.01 (0.94-1.09), respectively. The absence of recovery differences between the three groups remained analysing children and adults separately (data not shown).

Table 4-5 Clinical outcomes comparing the controls, Groups A (20 mg/L) and B (40 mg/L) at Day 5 and Day 14 of the follow-up, overall and per age category (adults defined as ≥12 years of age) in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Controls	Group A	p-value	Group B	p-value
Overall					
Persistent symptoms at Day 5, n (%)	108/374 (28.9)	85/373 (22.8)	0.715	105/383 (27.4)	0.287
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.552	1 (1-1)	0.252
Documented fever at Day 5, n (%)	37/379 (9.8)	26/377 (6.9)	0.154	33/385 (8.6)	0.568
Elevated CRP at Day 5, n (%)	96/403 (23.8)	80/403 (19.9)	0.173	64/404 (15.8)	0.004
Persistent symptoms at Day 14, n (%)	19/403 (4.7)	23/403 (5.7)	0.497	25/404 (6.2)	0.420
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	0.516	1 (1-1)	0.556
Documented fever at Day 14, n (%)	9/378 (2.4)	12/373 (3.2)	0.487	9/392 (2.3)	0.938
Unscheduled visits, n (%)	16/403 (4.0)	13/403 (3.2)	0.570	21/404 (5.2)	0.404
Hospitalisation, n (%)	1/403 (0.25)	4/403 (1.0)	0.178	3/404 (0.7)	0.317
Children					
Persistent symptoms at Day 5, n (%)	52/185 (28.1)	41/186 (22.0)	0.178	47/195 (24.1)	0.374
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.272	1 (1-1)	0.320
Documented fever at Day 5, n (%)	17/188 (9.0)	17/188 (9.0)	1.000	20/195 (10.3)	0.688
Elevated CRP at Day 5, n (%)	36/203 (17.7)	34/204 (16.7)	0.775	24/204 (11.8)	0.089
Persistent symptoms at Day 14, n (%)	8/186 (4.3)	8/182 (4.4)	0.965	10/196 (5.1)	0.712
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	1.000	1 (1-1)	0.421
Documented fever at Day 14, n (%)	3/187 (1.6)	6/183 (3.3)	0.296	6/198 (3.0)	0.355
Unscheduled visits, n (%)	8/203 (3.9)	6/204 (2.9)	0.580	15/204 (7.4)	0.136
Hospitalisation, n (%)	0/203 (0)	3/204 (1.5)	0.083	3/204 (1.5)	0.083
Adults					
Persistent symptoms at Day 5, n (%)	56/189 (29.6)	44/187 (23.5)	0.181	58/188 (30.9)	0.796
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.181	1 (1-1)	0.481
Documented fever at Day 5, n (%)	20/191 (10.5)	9/189 (4.8)	0.036	13/190 (6.8)	0.208
Elevated CRP at Day 5, n (%)	60/200 (30.0)	46/199 (23.1)	0.120	40/200 (20.0)	0.021
Persistent symptoms at Day 14, n (%)	11/190 (5.8)	15/189 (7.9)	0.408	15/194 (7.7)	0.449
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	0.459	1 (1-1)	0.246
Documented fever at Day 14, n (%)	6/191 (3.1)	6/190 (3.2)	0.993	3/194 (1.6)	0.300
Unscheduled visits, n (%)	8/200 (4.0)	7/199 (3.5)	0.800	6/200 (3.0)	0.586
Hospitalisation, n (%)	1/200 (0.5)	1/199 (0.5)	0.997	0/200 (0)	0.317

Severity based on patient's declaration and ranged between 1 (mild severity) to 4 (very severe symptoms); CRP: C-reactive protein; Elevated CRP defined as CRP ≥50 mg/L in children and ≥100 mg/L in adults; Unscheduled visits at the CRP Study primary care centre where the patient was recruited.

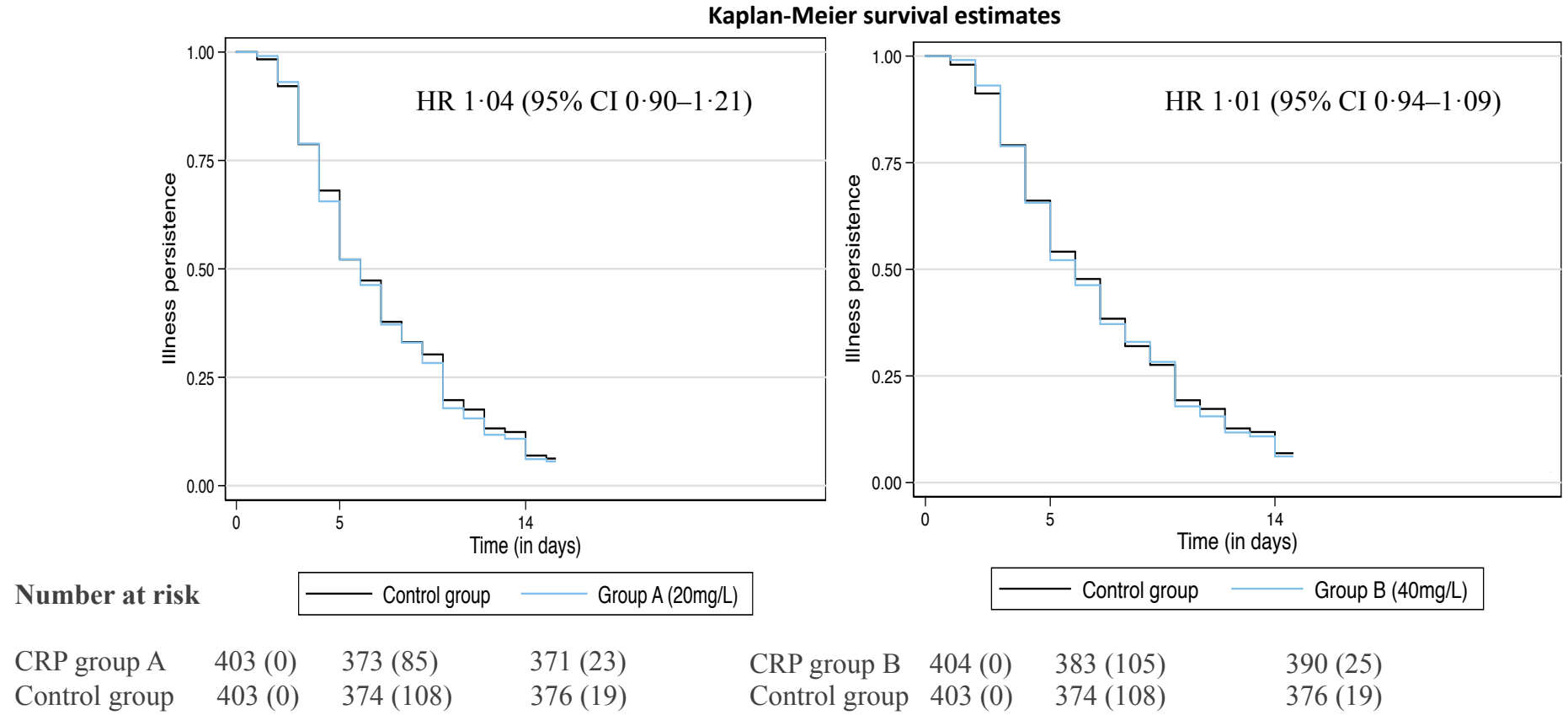


Figure 4-5 Kaplan-Meier curves of symptoms duration in the controls *versus* Groups A (20 mg/L) and B (40 mg/L) in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

The *per-protocol* analysis found similar clinical outcomes between the three different groups overall, and among children and adults separately, as indicated in Table 4.6. From the 968 children and adults included in the *per-protocol* analysis, symptom persistence at Day 5 was of 27.8% (104/374), 21.7% (63/291) and 25.4% (77/303) and at Day 14 of 5.1% (19/374), 6.5% (19/291) and 5.3% (16/303) in the controls, Group A and Group B, respectively (p-value ≥0.05). Figure 4.6 graphically presented survival estimates. The corresponding Log-rank test (p-value 0.568) and HR (1.04 [0.89-1.22]) did not identify any significant differences between the controls and Group A, as well as with Group B (p-value 0.373 and HR 1.03 [0.96-1.12]). The absence of difference in recovery was maintained analysing children and adults separately (data not shown).

Table 4-6 Clinical outcomes in the *per-protocol* analysis comparing the controls, Groups A (20 mg/L) and B (40 mg/L) at Day 5 and Day 14 of the follow-up, overall per age category in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Controls	**Group A**	**p-value**	**Group B**	**p-value**
Overall	*n=374*	*n=291*		*n=303*	
Persistent symptoms at Day 5, n (%)	104 (27.8)	63 (21.7)	0.062	77 (25.4)	0.435
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.487	1 (1-1)	0.387
Documented fever at Day 5, n (%)	24 (6.4)	13 (4.5)	0.230	16 (5.3)	0.399
Elevated CRP at Day 5, n (%)	7 (1.9)	4 (1.4)	0.576	2 (0.7)	0.139
Persistent symptoms at Day 14, n (%)	19 (5.1)	19 (6.5)	0.428	16 (5.3)	0.904
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	0.601	1 (1-1)	0.833
Documented fever at Day 14, n (%)	8 (2.1)	8 (2.8)	0.777	7 (2.3)	0.967
Unscheduled visits, n (%)	13 (3.5)	10 (3.4)	0.978	17 (5.6)	0.180
Hospitalisation, n (%)	1 (0.3)	2 (0.7)	0.423	2 (0.7)	0.444
Children					
Persistent symptoms at Day 5, n (%)	50/185 (27.0)	30/145 (20.7)	0.167	32/156 (20.5)	0.137
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.272	1 (1-1)	0.320
Documented fever at Day 5, n (%)	11/185 (6.0)	9/145 (6.2)	0.940	11/156 (7.1)	0.707
Elevated CRP at Day 5, n (%)	2/169 (1.2)	2/172 (1.2)	0.986	1/181 (0.6)	0.522
Persistent symptoms at Day 14, n (%)	8/185 (4.3)	7/145 (4.8)	0.825	7/156 (4.5)	0.930
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	1.000	1 (1-1)	0.421
Documented fever at Day 14, n (%)	5/185 (2.7)	5/145 (3.5)	0.858	6/156 (3.9)	0.629
Unscheduled visits, n (%)	5/185 (2.7)	3/145 (2.1)	0.710	14/156 (9.0)	0.012
Hospitalisation, n (%)	0 (0)	2 (1.4)	0.109	2 (1.3)	0.122
Adults					
Persistent symptoms at Day 5, n (%)	54/189 (28.6)	33/146 (22.6)	0.209	45/147 (30.6)	0.700
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.181	1 (1-1)	0.481
Documented fever at Day 5, n (%)	13/189 (6.9)	4/146 (2.7)	0.065	5/147 (3.4)	0.096
Elevated CRP at Day 5, n (%)	5/145 (3.5)	4/157 (2.6)	0.646	3/163 (1.8)	0.376
Persistent symptoms at Day 14, n (%)	11/189 (5.8)	12/146 (8.2)	0.397	9/147 (6.1)	0.917
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-1)	0.459	1 (1-1)	0.246
Documented fever at Day 14, n (%)	3/189 (1.6)	3/146 (2.1)	0.832	1/147 (0.7)	0.375
Unscheduled visits, n (%)	8/189 (4.2)	7/146 (4.8)	0.805	3/147 (2.0)	0.263
Hospitalisation, n (%)	1/189 (0.5)	0 (0)	0.379	0 (0)	0.377

Severity based on patient's declaration and ranged between 1 (mild severity) to 4 (very severe symptoms); CRP: C-reactive protein; Elevated CRP defined as CRP ≥50 mg/L in children and ≥100 mg/L in adults; Unscheduled visits at the CRP Study primary care centre where the patient was recruited.

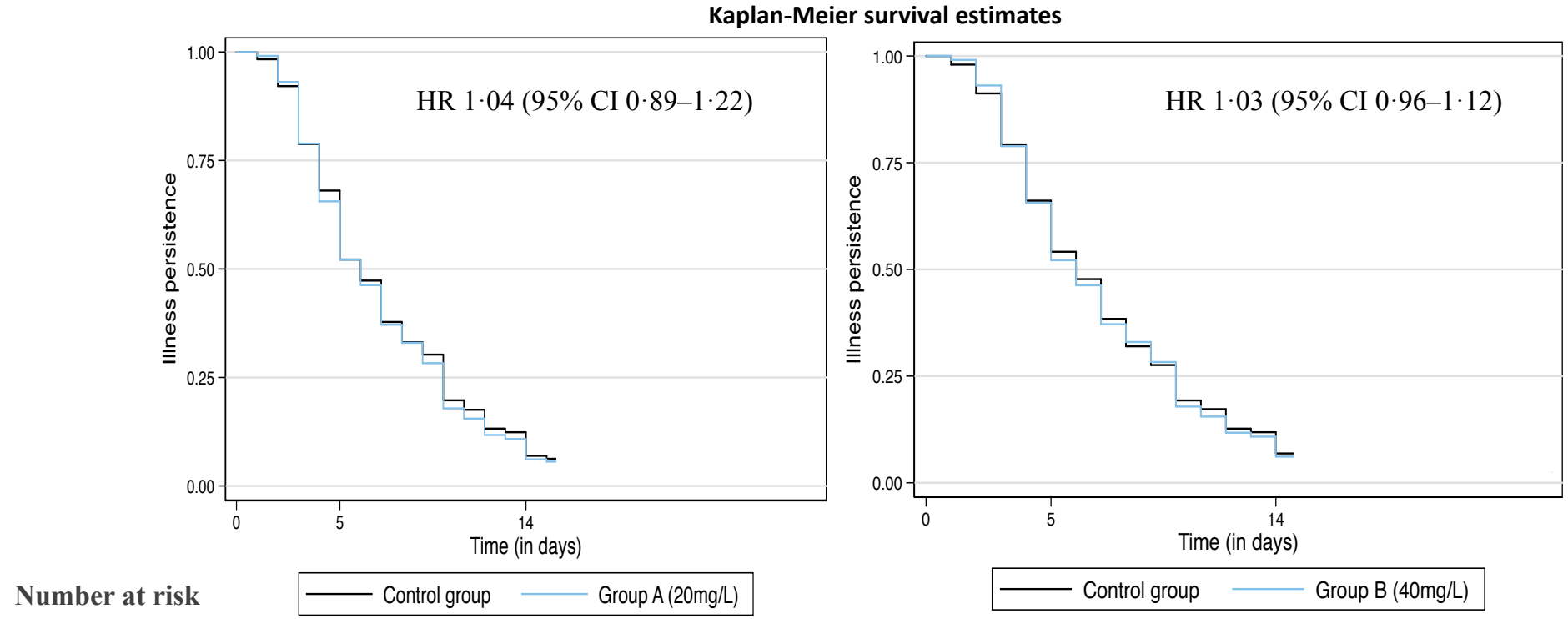


Figure 4-6 Kaplan-Meier curves of symptoms duration in the controls *versus* Groups A (20 mg/L) and B (40 mg/L) in a *per-protocol* analysis in Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Among 1,228 patients enrolled, 8 serious adverse events (SAEs) occurred (hospitalisations without any death): two in Group A, four in Group B and two in the controls (p-value 0.801 for Group A and 0.278 for Group B).

Among these eight SAEs, six were classified as "not related" to the study and two were "possibly related": the first one occurred in a two-year-old female presenting with a non-febrile diarrhoea with "low CRP" (CRP 30 mg/L in Group B), initially diagnosed by the clinic health worker as an "acute dysentery" and no antibiotic was prescribed. The patient was hospitalised four days after enrolment for persistent symptoms aggravated by a psychomotor slowing. The final diagnosis at the hospital was "acute gastro-enteritis" with prescription of an oral rehydration solution, folic acid, Zinc and a probiotic. No antibiotic was prescribed and the patient was discharged 24 hours after admission. The second "possibly related" SAE consisted of a five-year-old male randomised in Group A ("low CRP", 17 mg/L), with an initial diagnosis of common cold. No antibiotic was prescribed at patient enrolment. This patient was hospitalised 24 hours after study inclusion because of the persistence of symptoms, and was discharged three days later with the final diagnosis of "acute viral infection". Antibiotics were administered to this patient, including 3rd generation cephalosporins (Ceftriaxone IV and Cefixime PO), folic acid, anti-pyretic (paracetamol PO) and an anti-histaminic (Cetirizine).

4.3 Discussion

4.3.1 The baseline survey

The baseline survey was important for interpreting our trial results, as the research environment is not comparable to routine care. In the particular context of the CRP Study, we provided an additional diagnostic tool, as well as information and education about the management of fever and the risk of antibiotic overuse. Moreover, our research staff was constantly present on site during the whole study period, documenting the routine healthcare workers prescribing. Our intervention was mainly behavioural, as we intended to change prescribing practices towards febrile patients, and all these components were likely to modify routine prescribing behaviour.

The design of the study probably affected standard-of-care even further: health workers were alternately exposed to the CRP POCT and controls within the same facility, while most patients had "low" CRP concentrations, with the recommendation to withhold antibiotic. The least severe patients presenting with a self-limiting infection are likely to present with low inflammatory levels, and this relation may have been noticed during the study. It is therefore likely that health workers withheld antibiotics for patients randomised in the control group and presenting with a mild presentation. Additionally, three of our four sites in Myanmar belong to MAM, with repeated clinical trainings and extensive experience in febrile illness including malaria, HIV and TB (http://medicalactionmyanmar.com). Although antibiotic data from these three clinics were not available by febrile status, it is possible that routine prescription levels are lower than public primary health centre in Myanmar.

In the baseline survey, routine prescription levels were found to be high, varying between 41.1% among all patients (including non-febrile) in the three MAM clinics, to 68.8% among patients with a documented fever in Hlaing Tha Yar hospital outpatient department. This is consistent with a 2014 report in Myanmar primary care, where prescription levels were measured at 47% among all attending patients and reached 87% among those with a documented infection [34]. In more detail, MAM clinics showed a broad range of prescription levels between 32 and 41%, although being part of the same

network with similar clinical training. This heterogeneity has previously been reported both between primary health centres and healthcare workers within the same health facility, which indicates a potential for strengthening the consistency of patient management [321-323].

4.3.2 Impact on antibiotic prescribing

Despite potential biases and heterogeneity in prescribing behaviours described above, we demonstrated a significant antibiotic reduction using a 40 mg/L CRP threshold without altering clinical recovery. This reduction was marked when considering patients with a documented fever, as well as among patients with a respiratory presentation. The latter finding should be highlighted, as acute respiratory tract infection (ARI) is the most common reason for attending primary levels of care [242, 352, 353]. The reduction extended to patients with a prescription of broad-spectrum antibiotics, while these are a major contributor of bacterial resistance [354]. All these reductions were of lesser magnitude and mostly not of statistical significance between Group A and the control group.

Although significant, antibiotic reduction was of a lesser intensity than we anticipated: in our protocol, we expected a routine prescription of 70%, with a reduction of 20 percentage points to 50%, whereas antibiotic prescription was only 42.2% among controls and reduced by 7.8 percentage-points in the intervention Group B. We described the potential impact of the Hawthorne effect as well as the contamination of the control group on prescribing behaviour, and future studies limiting the presence of research staff onsite, combined with a cluster design where facilities and not patients are randomised should be envisaged. Nevertheless, antibiotic reduction was modest in our study, and it is challenging to quantify the impact of such reduction on the development of bacterial resistance. Primary care reaches the largest proportion of the general population [236], and is the greatest source of antibiotic use in humans [355]. Therefore, reducing antibiotic use, even by a few percentage-points, may mitigate drug pressure and the subsequent emergence of bacterial resistance.

During the CRP Study, lower than baseline prescription levels among the controls undermined the impact of CRP POCT on antibiotic reduction. In the literature, low or non-significant reduction occurred when routine prescription levels were already low, while great reductions have been reported when CRP is used for high prescription baseline. A large review among primary care patients with an

ARI in high-income countries showed the greatest reductions when antibiotic prescriptions among control groups were 54.5%, 83.0%, and 68.3% in Cals et al., 2013, Llor et al., 2012 and Cals et al., 2011, respectively) [264]. On a similar population, the review from Aabenhus et al. (2014) found CRP to reduce the risk of antibiotic prescription by 0.78 (95% CI 0.66-0.92) with control groups being prescribed in 51.9% of cases in Melbye et al., 1995, 46.2% in Diederichsen et al., 2000, and 56.6% in Cals et al., 2010 [79]. In our trial, prescription rate in the control group was lower than most of these studies in primary care, only reaching 42.2% and therefore weakening the impact of CRP on antibiotic reduction.

The impact of CRP-testing was presented only in Myanmar in this Thesis. However, a comparison with the Thai sites in Chiang Rai may bring additional insights on the importance of the context on this type of intervention. In Thailand for instance, several antibiotic stewardship programmes have been implemented under the supervision of the Ministry of Public Health (MoPH): in 2007, the Antibiotic Smart Use (ASU) programme sets a target prescription rate of 20% per month for respiratory infections and acute diarrhoea, intended to public primary care units (PCUs) [356]. This programme included financial incentives at the facility level. During the CRP Study in August 2016, the National Strategic Plan on antimicrobial resistance 2017-2021 was endorsed within the national Thai strategy, and added incentives to MoPH health inspectors to supervise hospitals and PCUs' antibiotic prescription targets [289]. On the contrary, Myanmar did not set any programme to achieve appropriate use of antimicrobial in humans, at least until 2015 [34]. This contrast in policy environment may partially explain differences we observed in prescribing behaviours between control groups from Thailand and Myanmar (at Day 0, 32.1% [127/396] in Thailand *versus* 42.2% [170/403] in Myanmar, p-value <0.001). This difference in routine prescription impacted our intervention, CRP POCT reducing antibiotic prescription by 3.1 percentage-points (RD -3.1 [-0.9, 2.5]) in Thailand, compared to 6.6 percentage-points (RD -6.6 [-12.5, -0.8]) in Myanmar at Day 0.

Our study evaluated the impact of CRP-testing in isolation. Additional interventions for reducing antibiotic prescription alongside CRP-testing have been evaluated: a multinational cluster randomised controlled trial demonstrated the impact of a training in communication skills, by improving health worker's understanding of patient's concerns and expectations while ensuring a good

comprehension of the treatment objective [119]. Another objective was to address the potential options available in case of doubt for a prescription, by encouraging re-consultations. This training also demonstrated to significantly reduce antibiotic prescriptions even without testing for CRP. Accompanying the provision of the CRP test with a training for facilitating its interpretation and the relevant treatment options was also proven to be more effective than solely providing the test alone [119]. In our study, health workers benefited from training at the beginning and at study mid-point, which may have lowered routine prescribing levels in the control group further.

Another strategy may be to integrate CRP-testing in a prescriptive algorithm, potentially within a package of interventions. In Tanzania, a primary care-based study assessed a combination of CRP, procalcitonin, pulse oximetry, glucometer and haemoglobin tests, using an electronic point-of-care algorithm (e-POCT) in febrile children, and reduced antibiotic prescription from 95% to 10% [99]. Several comments though: the costs of these interventions may be unrealistic regarding the resource constraints in LMICs; a strict compliance to the e-POCT at the expense of clinical judgment may not be acceptable in routine care; such reductions occurred with an unusually high baseline prescription level (95% in this Tanzanian study *versus* 42.2% in the CRP Study).

Antibiotic reduction should not be the only highlighted outcome: antibiotic coverage was greater among patients with "high" CRP levels in the intervention groups than in the controls. Health workers complied when CRP-results were considered "high" (in 72.8% when CRP >20 mg/L and in 75.9% when CRP >40 mg/L), indicating that CRP may represent an additional safety layer by covering a potential bacterial infection. Only 43.3% of patients in the control group had an antibiotic prescribed for CRP levels greater than 40 mg/L, compared to 72.7 and 78.6% in Groups A and B. Systemic bacterial infections are associated with high inflammatory levels, and although patients with CRP concentrations greater than 40 mg/L may not all require an antibiotic, ensuring antibiotic coverage among patients living in hard-to-reach areas may be a safe approach [357]. A 2018 study showed that 86.1% of confirmed-bacterial infections were characterised by CRP levels greater than 40 mg/L, in a Chiang Rai province where some of the CRP Study sites were based. [110]. Primary care patients are not severe by definition, with a low pre-test probability for a serious bacterial infection, and the majority

of our patients had low CRP levels (72% with CRP <20 mg/L and 86% with CRP <40 mg/L at Day 0; Figure 4.7).

This may imply that most primary care patients do not suffer from a serious bacterial infection, and therefore do not require an antibiotic. Most studies in Southeast Asia selected thresholds ranging between 10-40 mg/L, CRP showing high sensitivity but only moderate specificity in the detection of bacterial infections, implying a very low likelihood for missing a systemic bacterial infection [107, 110]. The use of a high CRP threshold of 40 mg/L did not show any difference in clinical recovery compared to the controls and may be appropriate for these non-severe patients, while a stricter compliance to test results would reduce prescriptions to a larger extent, as presented in the *per-protocol* analysis (>20 percentage-point reduction between Group B and the controls; Table 4.3).

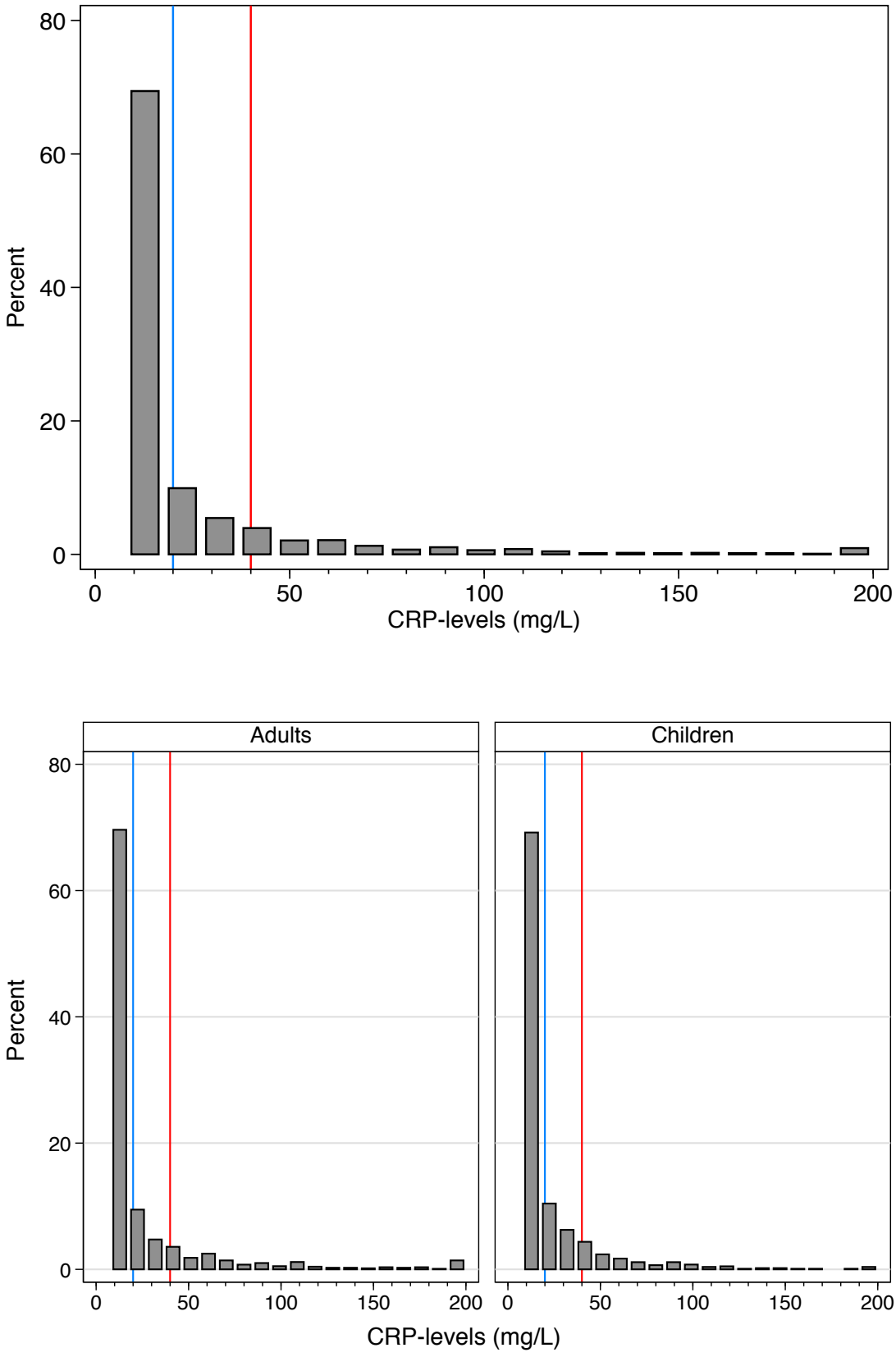


Figure 4-7 CRP distribution overall and per age category

In our study, health workers had a concomitant access to CRP-results at the time of the physical examination. Recent evidence, all from high-income countries, evaluated whether CRP-testing should be restricted to febrile patients deemed at risk of a serious infection based on a preliminary clinical assessment: in Belgium, a cluster randomised controlled trial reduced the number of CRP-tested patients without reporting any differences in hospital referral for a serious infection compared to children who were systematically tested for CRP [358]. In Norway, neither antibiotic prescription nor hospital referral were significantly reduced regardless of whether CRP-testing was used amongst all children with respiratory symptoms or restricted to doctor's indication [286]. The risk for an invasive bacterial infection is limited in primary care, and both Belgium and Norwegian referral systems are well-structured with regularly-trained medical doctors, which is illustrated by low prescription levels in routine practice (e.g. 22% in the Norwegian control group). In this particular context, it is therefore possible that selective CRP-testing is relevant. However, such a strategy may not be safe in LMICs where clinical judgment is variable and health worker face the double constraint of high infectious diseases burden and risk of loss to follow-up with unreliable referral structures [34, 35, 234].

4.3.3 Clinical outcomes

Secondary outcomes included clinical recovery of our patients, based on a medical examination both at Day 5 and at Day 14 of the follow-up, as well as measurement of CRP at Day 5, occurrence of any unscheduled visit to the facility and SAE. We did not detect any differences in all these outcomes across the three groups, suggesting a safe antibiotic reduction, which is consistent with previous studies including in LMICs [211, 274] and high-income countries [79, 264]. Furthermore, CRP has been shown to be of high sensitivity in detecting bacterial infections in Southeast Asia, implying that most infections requiring an antibiotic are covered [107].

Several caveats should be mentioned: no studies -including ours- were powered to prove safety in antibiotic reduction. The occurrence of adverse events is rare in primary care where patients are non-severe by definition, therefore, demonstrating the absence of negative outcomes from an antibiotic reduction would require a large sample size combined with a clinical assessment to ascertain patient recovery. In Tanzania, Keitel et al., was the first to demonstrate safety in reducing antibiotic using CRP,

recruiting no less than 1,726 febrile children with non-severe respiratory symptoms [211]. As discussed previously, CRP-testing was not the only intervention but combined with several additional diagnostic tools within an electronic algorithm, and repeated clinical training and supervision alongside the study. Besides, most studies from both high-income and low- to middle-income countries limited their evaluation to patients with respiratory symptoms. All this indicates that safety of CRP-testing for reducing antibiotic prescription has yet to be ascertained in more pragmatic conditions, and extended to all febrile patients.

Finally, clinical outcome was measured using face-to-face interviews including a medical examination 14 days after enrolment, but some patients only received a phone interview due to their inability to attend the facility. Measuring a clinical outcome via a phone interview may represent a limitation to prove safety, undermining the interpretation of this outcome. In the literature, patient recovery is sometimes measured by re-consultations [114, 282, 285, 288, 301], which poses the question about patients potentially seeking health care in a different facility in case of symptom worsening or persistence. However, most studies follow patients for more than one week, more generally between 14 to 30 days, which allows capture of potential relapses [79, 211, 264, 274].

Chapter 5

THE CRP STUDY – CAUSES OF FEVER AND PERFORMANCE OF C-REACTIVE PROTEIN IN DISCRIMINATING BACTERIAL FROM VIRAL PATHOGENS

5 THE CRP STUDY – CAUSES OF FEVER & PERFORMANCE OF C-REACTIVE PROTEIN IN DISCRIMINATING BACTERIAL FROM VIRAL PATHOGENS

5.1 Research in context

Fever is a common reason for seeking healthcare in Southeast Asia and as malaria incidence declines, bacteria and viruses now represent the main contributors to febrile illness [3, 5-7, 15]. Primary care in low-to middle-income countries (LMICs) is typically characterised by a shortage in human resources, diagnostics and evidence-based guidelines [25]. Studies investigating causes of fever in this environment are few and frequently of poor quality: enrolment is often limited to a single clinical presentation and specific age category, and microbiological investigations rarely use gold standard methods [21, 359, 360]. Additionally, most primary care patients attend early after symptom onset with non-severe presentations, lowering the chances of detecting a pathogen [17-21, 23, 24, 359, 360]. Empiric treatment guidelines are therefore based on limited epidemiological evidence and are often implemented by insufficient and poorly trained staff, contributing to irrational antibiotic prescription practices [28-30]. Furthermore, clinical presentations often overlap between bacterial and viral infections, encouraging prescribers to choose the safest option, which is to prescribe an antibiotic [6, 7, 31].

Given these limitations in clinical judgment and laboratory structures, point-of-care testing (POCT) to guide fever management could be beneficial in primary care [101]. C-reactive protein (CRP) is one of the most studied host-response biomarkers of bacterial infection, consistently showing high sensitivity and moderate specificity [102, 361]. However, 80% of CRP performance evaluations originate from high-income countries [361]. In Southeast Asia, these evaluations are mainly hospital-

based [82, 83, 110] with only one community-based study [107]. Good diagnostic performance of CRP in identifying bacterial infections was observed but generalisability was limited due to demographic, clinical and diagnostic heterogeneity of these studies.

Using the CRP Study, we aim to identify key organisms among acutely febrile children and adults attending primary care in Chiang Rai, northern Thailand, and Hlaing Tha Yar, Lower Myanmar, and to evaluate the performance of CRP for discriminating between bacteria and viruses [362].

5.2 Results

5.2.1 Baseline characteristics

Of the 799 febrile patients prospectively enrolled and randomised into the CRP Study control group, 773 (96.8%) had at least one blood or nasopharyngeal (NP) swab specimen collected, including 371 (48.0%) children and 402 adults (52.0%). Specimens were collected throughout dry and wet seasons, from June 2016 to August 2017. As presented in Table 5.1, children presented significantly earlier after symptom onset than adults, with fewer comorbidities and less self-reported medication (p-value <0.05). Antibiotic intake declaration was similar in children and adults (p-value 0.237), but significantly more frequent in Thailand than in Myanmar (27/396 [6.8%] *versus* 14/403 [3.5%], p-value <0.001, respectively).

Clinically, respiratory tract symptoms were the most prevalent presentation both in children and adults. Runny nose, sore throat or cough were the most frequent symptoms, while upper respiratory tract infection (URTI) accounted for the most frequent diagnosis among patients with respiratory tract symptoms in children and adults (91.9% and 91.3%, p-value 0.794, respectively). Within URTI, common cold was diagnosed in 48.2% (120/249) of children and 45.0% (108/240) of adults (p-value 0.479). Gastrointestinal symptoms were the second most prevalent presentation, and there were no differences between children and adults. Children presented with a documented fever more frequently than adults, but with less neurological complaints.

Table 5-1 Baseline characteristics of children and adults in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Children (n=371)	Adults (n=402)	p-value
Demographic characteristics			
Age (in years), median (IQR)	5 (3-8)	33 (22-52)	
Female, n (%)	179 (48.3)	246 (61.2)	<0.001
Comorbidities, n (%)	11 (3.0)	111 (27.6)	<0.001
Onset of symptoms (in days), median (IQR)	2 (1-3)	3 (2-4)	<0.001
Self-reported medication intake, n (%)	232 (62.5)	290 (72.1)	0.004
Self-reported antibiotic intake, n (%)	16 (4.3)	25 (6.2)	0.237
Clinical characteristics			
Documented fever, n (%)	176 (47.4)	153 (38.1)	0.007
Neurological symptoms, n (%)	62 (16.7)	146 (36.3)	<0.001
Respiratory tract symptoms, n (%)	271 (73.1)	263 (65.4)	0.022
- URTI, n (%)	249 (67.1)	240 (59.7)	0.033
Gastro-intestinal symptoms, n (%)	99 (26.7)	95 (23.6)	0.328
Other symptoms, n (%)	33 (8.9)	25 (6.2)	0.170
Biological characteristics			
Blood specimen available, n (%)	338 (91.1)	402 (100.0)	<0.001
Total number of organisms detected in blood, n (%)	49/338 (14.5)	30/402 (7.5)	0.002
- Bacterial organisms, n (%)	15/338 (4.4)	18/402 (4.5)	0.398
- Viral organisms, n (%)	34/338 (10.1)	12/402 (3.0)	0.010
Nasopharyngeal swab available, n (%)	268 (72.2)	359 (89.3)	<0.001
Total number of swabs ≥1 organism detected, n (%)	230/268 (85.8)	238/359 (66.3)	<0.001
Total number of organisms per swab, median (IQR)	3 (1-4)	1 (0-1)	<0.001
- Bacterial organisms per swab, median (IQR)	2 (1-3)	0 (0-1)	<0.001
- Viral organisms per swab, median (IQR)	1 (0-2)	1 (0-1)	<0.001
Mixed bacterial - viral organisms in swabs, n (%)	155/268 (57.8)	57/359 (15.9)	<0.001

Comorbidities included HIV, hepatitis B or C, cirrhosis, diabetes mellitus, asthma, anaemia, chronic obstructive pulmonary disease, congenital heart or kidney disease, alcoholism, dyslipidaemia, G6PD deficiency, hypertension, rheumatic heart disease, thalassaemia, thyroid disease
Neurological symptoms include headache, confusion, or hearing loss.
Respiratory tract symptoms included sore throat, dyspnoea, chest pain, runny nose, or cough
URTI (upper respiratory tract infection) was defined by the presence of these symptoms only: runny nose, sore throat or cough.
Gastro-intestinal symptoms included nausea, vomiting, jaundice, diarrhoea, or abdominal pain.
Other symptoms declared were defined by the presence of fever alone or symptoms other than those present in neurological, respiratory, or gastrointestinal symptoms. Common symptoms in this group included myalgia, arthralgia, tiredness, chills, sweating, weight loss, skin eruption, dysuria, dizziness or eye redness.
The prescription of antibiotics at the facility was considered between the enrolment at day 0 until day 14 of the follow-up

5.2.2 Organisms detected

A blood specimen was available in 91.1% (338/371) of children and all 402 adults (total n=740). Organisms were detected in blood more frequently in children than adults (49/338 [14.5%] *versus* 30/402 [7.5%], p-value 0.002, respectively). Among the 740 patients with a blood specimen available, 79 (10.7%) had an organism detected including 50/347 (14.4%) in Myanmar and 29/393 (5.0%) in Thailand (p-value 0.007).

Bacteria and virus distribution in blood is provided across age categories in Table 5.2: the most frequent bacterium was *Leptospira* spp. accounting for nine cases (two children, seven adults), followed by *Klebsiella pneumoniae* (eight cases; five children and three adults), *Streptococcus suis* (four cases; two children and two adults) and *Rickettsia* spp. (three cases; two children and one adult). The most common virus was dengue with 30 cases (21 children, 9 adults), followed by enterovirus (8 cases; 7 children and 1 adult) and rhinovirus (4 cases; all children). One child had both *Rickettsia* spp. and *Salmonella* Paratyphi A, detected by the TAC assay. Corresponding patient characteristics are detailed in Table 5.3.

Nasopharyngeal swabs were available in 268/371 (72.2%) children and 359/402 (89.3%) adults (total n=627). Organisms were detected more frequently and with a higher median number among children compared with adults (230/268 [85.8%] *versus* 238/359 [66.3%] and 3 [interquartile range 1-4] *versus* 1 [0-1], p-value <0.001, respectively). The number of organisms per swab remained higher among children when analysing bacteria and viruses separately (p-value <0.001). Similarly, co-detection of bacteria and viruses were more frequent in children than adults (155/268 [57.8%] *versus* 57/359 [15.9%], p-value <0.001, respectively).

According to the criteria described in the Methods section and based on the literature review, an organism detected in NP swabs was considered pathogenic among 177 out 627 patients (28.2%), including 67 out of 268 children (25.0%) and 110 out 359 (30.6%) adults. Influenza virus type A was the most prevalent target organism with 85 cases out of 627 NP swabs tested (13.6%), followed by respiratory syncytial virus (RSV) detected in 24 cases (3.8%), Influenza virus type B with 22 cases (3.5%), human metapneumovirus (hMPV) with 16 cases (2.6%), and *Bordetella pertussis* with three paediatric cases (0.5%).

Table 5-2 Bacterial and viral detection in blood and nasopharyngeal swabs specimens among children and adults in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Blood		**Nasopharyngeal swabs (n=627)**	
	Children	Adults	Children (n=268)	Adults (n=359)
Bacteria				
Aerococcus spp. (n =630) *	0	1/401 (0.3%)	-	-
Acinetobacter baumannii	-	-	1 (0.4%)	3 (0.8%)
Bordetella parapertussis	-	-	0	0
Bordetella pertussis	-	-	**3 (1.1%)**	**0**
Corynebacterium diphtheriae	-	-	1 (0.4%)	0
Chlamydia trachomatis	-	-	0	0
Chlamydophila pneumoniae	-	-	1 (0.4%)	3 (0.8%)
Group A *Streptococcus* (n = 630)	0	0	6 (2.2%)	1 (0.3%)
Haemophilus influenzae (n = 630)	1/229 (0.4%)	0	121 (45.2%)	42 (11.7%)
Klebsiella pneumoniae (n = 630)	5/229 (2.2%)	3/401 (0.8%)	13 (4.9%)	19 (5.3%)
Leptospira spp. (n = 634) **	2/232 (0.9%)	7/402 (1.7%)	-	-
Moraxella catarrhalis	-	-	124 (46.3%)	31 (8.6%)
Mycoplasma pneumoniae	-	-	1 (0.4%)	0
Pseudomonas aeruginosa	-	-	2 (0.8%)	1 (0.3%)
Orientia tsutsugamushi (n = 658) ***	0	0	-	-
Rickettsia spp. (n = 658)	3/256 (1.2%)	1/402 (0.3%)	-	-
Salmonella spp. (n = 630)	0	2/401 (0.5%)	-	-
Salmonella Paratyphi A (n = 630)	2/229 (0.9%)	1/401 (0.3%)	-	-

Staphylococcus aureus (n = 630)	0	0	44 (16.4%)	27 (7.5%)
Streptococcus pneumoniae (n = 630)	0	0	130 (48.5%)	34 (9.5%)
Streptococcus suis (n = 630)	2/229 (0.9%)	2/401 (0.5%)	-	-
Streptococcus spp. (n = 630)	0	1/401 (0.3%)	-	-
Viruses				
Adenovirus (n = 601) ****	0	0	13 (4.9%)	6 (1.7%)
Bocavirus (n = 601)	0	1/393 (0.3%)	12 (4.5%)	0
Chikungunya virus (n=686) *****	0	0	-	-
Dengue virus (n = 686)	21/290 (7.2%)	9/396 (2.3%)	-	-
Zika virus (n=686)	0	0	-	-
Enterovirus (n = 601)	7/208 (3.4%)	1/393 (0.3%)	27 (10.1%)	16 (4.5%)
Rhinovirus (n = 601)	4/208 (1.9%)	0	82 (30.6%)	54 (15.0%)
Influenza virus type A	-	-	**16 (6.0%)**	**69 (19.2%)**
Influenza virus type B	-	-	**13 (4.9%)**	**9 (2.5%)**
Human coronavirus	-	-	20 (7.5%)	17 (4.7%)
Measles (n = 601)	0	0	1 (0.4%)	1 (0.3%)
Parainfluenza virus (1-3)	-	-	19 (7.1%)	5 (1.4%)
Human metapneumovirus	-	-	**12 (4.5%)**	**4 (1.1%)**
Rubella virus (n = 601)	0	1/393 (0.3%)	-	-
Respiratory syncytial virus	-	-	12 (4.5%)	12 (3.3%)
Cytomegalovirus	-	-	44 (16.4%)	4 (1.1%)
Varicella-Zoster virus (n = 601)	2/208 (1.0%)	0	3 (1.1%)	0

Organisms with a high likelihood of causality in nasopharyngeal swabs are in **bold**

* 630 blood specimens tested for bacterial screening, using the Taqman array card (TAC) assay (n=601) and bacterial singleplex polymerase chain reaction (PCR) (n=626)

** 634 blood specimens tested for *Leptospira* screening, using the TAC assay (n=601), the bacterial singleplex PCR (n=626) and the microagglutination test (n=134)

*** 658 blood specimens tested for *Orientia tsutsugamushi* and *Rickettsia* spp. screening, using the TAC assay (n=601), the bacterial singleplex PCR (n=626), and the indirect immmunofluoresence assay (n=656)

**** 601 blood specimens tested using the TAC assay only (n=601)

***** 686 blood specimens tested for dengue, chikungunya and zika virus screening, using the TAC assay (n=601), the viral singleplex PCR on fresh blood (n=626) and dried blood spot (n=245)

Table 5-3 Patient characteristics by organism detected in blood specimens in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Myanmar (n=50)	Methods	CRP (mg/L)	Age (years)	Comorbidity	Symptom onset (days)	Tympanic temperature	Clinical presentation	Health worker diagnosis	Antibiotic prescription
Aerococcus spp.	Singleplex PCR	15	22	Hepatitis B	3	36.7 ˚C	Neurological & Respiratory	URTI	No
Leptospira spp.	Singleplex PCR	8	8	No	2	37.0 ˚C	Undifferentiated	Acute viral infection	No
	Singleplex PCR	8	33	Asthma	4	37.6 ˚C	Undifferentiated	Acute viral infection	No
	TAC	62	15	No	2	39.2 ˚C	Respiratory	URTI	No
	MAT	63	29	No	3	38.6 ˚C	Respiratory & Digestive	URTI	Azithromycin
	Singleplex PCR	200	13	No	3	39.0 ˚C	Respiratory & Digestive	URTI	Amoxicillin
	Singleplex PCR	200	14	No	5	37.5 ˚C	Neurological & Digestive	-	No
Klebsiella pneumoniae	TAC	8	6	No	2	38.4 ˚C	Respiratory	URTI	No
	TAC	10	11	No	3	38.4 ˚C	Respiratory	URTI	No
	TAC	10	56	No	4	37.0 ˚C	Neurological	-	Amoxicillin
	TAC	18	25	HIV	6	37.4 ˚C	Undifferentiated	HIV infection stage I	No
	TAC	28	37	No	3	37.3 ˚C	Respiratory	Acute viral infection	Amoxicillin
	TAC	44	7	-	1	37.7 ˚C	Digestive	Acute viral infection	No
	TAC	53	8	No	3	37.5 ˚C	Digestive	-	No
	TAC	98	7	No	1	37.5 ˚C	Respiratory	URTI	No
Rickettsia genus	IFA	29	32	No	5	36.9 ˚C	Neurological & Digestive	Acute gastritis	No
Salmonella Paratyphi A	TAC	26	32	No	3	36.6 ˚C	Digestive	Acute viral infection	No
Streptococcus suis	TAC	16	12	No	4	37.9 ˚C	Respiratory	URTI	Amoxicillin
	TAC	200	27	No	7	37.2 ˚C	Respiratory	LRTI	Amoxicillin
Streptococcus spp.	Singleplex PCR	12	28	No	2	37.1 ˚C	Respiratory & Digestive	URTI	Amoxicillin
Bocavirus	TAC	97	43	No	5	38.8 ˚C	Respiratory	-	Amoxicillin

	Singleplex PCR	-	2	No	1	38.3 ˚C	Digestive	Acute gastroenteritis	Ciprofloxacin
	Singleplex PCR	-	2	No	2	40.4 ˚C	Digestive	Chronic suppurative otitis media	Cloxacillin
	Singleplex PCR	-	4	No	1	39.5 ˚C	Digestive	Food poisoning	Ciprofloxacin
	Singleplex PCR	-	4	No	2	39.6 ˚C	Neurological & Digestive	Acute viral infection	No
	Singleplex PCR	-	4	No	4	38.6 ˚C	Neurological, Respiratory & Digestive	URTI	No
	TAC	8	9	No	4	37.5 ˚C	Undifferentiated	Acute viral infection	Amoxicillin
	Singleplex PCR & TAC	8	8	No	2	37.7 ˚C	Digestive	Acute gastroenteritis	No
	Singleplex PCR & TAC	8	13	No	1	39.9 ˚C	Respiratory	URTI	No
	TAC	8	13	No	1	38.4 ˚C	Neurological	URTI	No
	Singleplex PCR & TAC	8	16	No	1	38.8 ˚C	Respiratory	Acute viral infection	No
	Singleplex PCR & TAC	8	20	No	1	38.8 ˚C	Neurological & Respiratory	URTI	Amoxicillin
	TAC	9	6	No	5	37.1 ˚C	Neurological, Respiratory & Digestive	Acute viral infection	Cephalexin
	Singleplex PCR & TAC	10	7	No	5	37.5 ˚C	Undifferentiated	-	No
Dengue virus	Singleplex PCR	10	13	No	5	38.4 ˚C	Respiratory & Digestive	URTI	Azithromycin
	Singleplex PCR & TAC	10	6	No	2	38.6 ˚C	Respiratory	LRTI	Amoxicillin
	TAC	11	11	No	1	39.2 ˚C	Neurological	-	No
	Singleplex PCR & TAC	14	21	No	7	39.7 ˚C	Respiratory	Acute viral infection	No
	Singleplex PCR & TAC	15	8	No	2	37.6 ˚C	Digestive	Acute viral infection	No
	Singleplex PCR & TAC	15	12	No	1	39.0 ˚C	Respiratory & Digestive	Acute viral infection	No
	Singleplex PCR & TAC	17	18	No	3	39.1 ˚C	Digestive	Acute viral infection	No
	Singleplex PCR & TAC	20	10	No	5	38.7 ˚C	Undifferentiated	LRTI	Amoxicillin
	Singleplex PCR & TAC	31	7	No	1	37.6 ˚C	Neurological & Digestive	Head trauma	No
	Singleplex PCR & TAC	32	7	No	1	40.0 ˚C	Respiratory	LRTI	Amoxicillin
	Singleplex PCR & TAC	32	9	No	2	37.6 ˚C	Respiratory & Digestive	Acute viral infection	No
	Singleplex PCR & TAC	36	7	No	1	39.2 ˚C	Undifferentiated	Acute viral infection	No
	Singleplex PCR & TAC	43	5	No	2	39.6˚C	Undifferentiated	URTI	No
	Singleplex PCR & TAC	48	25	No	5	38.1 ˚C	Neurological	Non-specific fever	Azithromycin
	Singleplex PCR & TAC	105	8	No	1	40.2 ˚C	Neurological	-	No
Enterovirus	TAC	8	6	No	3	37.6 ˚C	Respiratory & Digestive	URTI	Amoxicillin

Thailand (n=29)	Methods	CRP (mg/L)	Age (years)	Comorbidity	Symptom onset (days)	Tympanic temperature	Clinical presentation	Health worker diagnosis	Antibiotic prescription
Haemophilus	TAC	28	4	No	1	36.9 °C	Respiratory	Common cold	No
Leptospira spp.	MAT	30	≥12	No	1	36.8 °C	Neurological & Respiratory	Acute pharyngitis	Amoxicillin
	Singleplex PCR	112	64	Hypertension	4	37.8 °C	Respiratory & Digestive	Common cold	No
	Singleplex PCR	158	8	No	1	36.9 °C	Neurological, Respiratory & Digestive	Common cold	No
Rickettsia genus	Singleplex PCR	10	8	No	2	37.0 °C	Neurological, Respiratory & Digestive	Acute tonsillitis	Amoxicillin
	Singleplex PCR	28	7	No	1	37.9 °C	Respiratory & Digestive	Acute pharyngitis	Amoxicillin
Salmonella Paratyphi A	TAC	8	11	No	3	36.7 °C	Neurological & Respiratory	Common cold	No
	TAC	18	10	No	3	36.6 °C	Respiratory	Acute pharyngitis	Amoxicillin
Salmonella Paratyphi A	TAC	8	11	No	1	39.6 °C	Respiratory	Acute pharyngitis	Amoxicillin
Salmonella spp.	TAC	9	62	Hypertension	7	36.9 °C	Respiratory	Common cold	No
	TAC	14	20	No	2	36.7 °C	Neurological & Respiratory	Acute pharyngitis	No
Streptococcus suis	TAC	8	5	No	1	37.8 °C	Respiratory	Common cold	No
	TAC	20	9	No	2	36.4 °C	Respiratory & Digestive	Common cold	No
Dengue	Singleplex PCR & TAC	12	3	No	2	39.2 °C	Neurological, Respiratory & Digestive	Acute pharyngitis	Amoxicillin
	Singleplex PCR & TAC	23	9	No	2	38.6 °C	Neurological, Respiratory & Digestive	Fever of unknown	No
Enterovirus	TAC	10	12	No	1	39.1 °C	Neurological & Respiratory	Acute tonsillitis	Amoxicillin
	TAC	12	8	No	1	38.5 °C	Respiratory	Acute pharyngitis	Amoxicillin
	TAC	13	4	No	3	37.4 °C	Respiratory	Common cold	No
	TAC	19	5	No	3	35.7 °C	Neurological & Respiratory	Common cold	No
	TAC	20	6	No	1	36.9 °C	Undifferentiated	Acute pharyngitis	Amoxicillin
	TAC	32	9	No	1	37.2 °C	Undifferentiated	Acute tonsillitis	Amoxicillin
	TAC	47	1	No	2	37.0 °C	Respiratory	Acute pharyngitis	Amoxicillin
Rhinovirus	TAC	8	1	No	1	37.6 °C	Respiratory	Common cold	No
	TAC	8	2	No	1	37.0 °C	Respiratory	Common cold	No
	TAC	9	11	No	2	37.1 °C	Respiratory	Acute pharyngitis	Amoxicillin
	TAC	33	3	No	2	37.8 °C	Respiratory	Common cold	No
Rubella virus	TAC	8	15	No	3	36.7 °C	Neurological, Respiratory & Digestive	Acute tonsillitis	Amoxicillin
Varicella-Zoster virus	TAC	8	11	No	1	37.0 °C	Digestive	Common cold	No
	TAC	13	7	No	1	37.0 °C	Neurological	Varicella	Amoxicillin

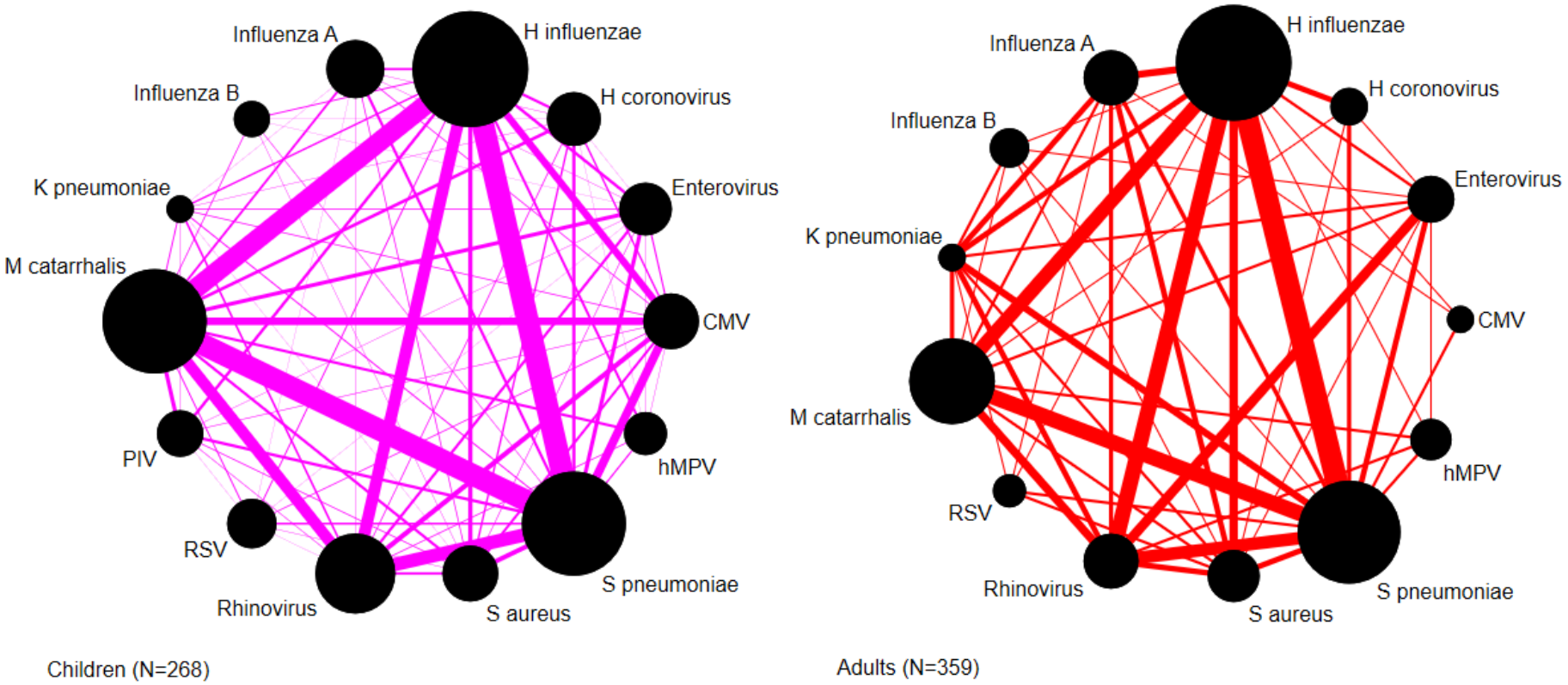


Figure 5-1 Network analysis for bacterial and viral detection and combination in nasopharyngeal swabs among children and adults in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

The size of the black dot represents the frequency of organism detection in nasopharyngeal swabs. Only organisms detected in more than 10 specimens are represented. Each of the axes between the dots represents the frequency of combination between two organisms.

Bacterial organisms: *K pneumoniae* is *Klebsiella pneumoniae; S pneumoniae* is *Streptococcus pneumoniae; M catarrhalis* is *Moraxella catarrhalis; S aureus* is *Staphylococcus aureus;* and *H influenzae* is *Haemophilus influenzae.*

Viral organisms: CMV is cytomegalovirus; H coronavirus is human coronavirus (229E; NL63; OC43 and HKU1; hMPV is human metapneumovirus; RSV is respiratory syncytial virus; and PIV is parainfluenza virus (type 1-3).

The distribution of bacteria and viruses in NP swabs per age category is illustrated in Figure 5.1, as well as co-detections, which were more common in children than adults.

5.2.3 Accuracy of current prescribing practices and CRP-guided treatment

Of the 227 patients with a pathogen detected, 36 (15.9%) were allocated to the bacterial group and 191 (84.1%) to the viral group. The median CRP concentration was higher in the bacterial group compared to the viral one (18 [10-49] mg/L *versus* 10 [≤8-22] mg/L, p-value 0.003, respectively). Among the 422 patients for whom no pathogenic organism was detected, CRP median was 10 (≤8-29) mg/L, lower than in the bacterial group (p-value 0.004) but similar to patients with a viral pathogen detected (p-value 0.826). CRP concentration distribution is presented by aetiological group in Figure 5.2.

The AUC for CRP distinguishing bacterial from viral pathogens was 0.652, 95% confidence interval (0.553-0.750), as illustrated in Figure 5.3.

The sensitivity of current prescribing practices for the detection of bacterial pathogens was 33.3% (95% CI 18.6-51.0%) and specificity was 60.2% (95% CI 52.9-67.2%). Using a threshold of 20 mg/L would imply an increase of sensitivity to 47.2% (95% CI 30.4-64.5%) and an increase in specificity to 68.9% (62.3-75.5%), respectively. Use of the higher threshold of 40 mg/L would imply a lower sensitivity of 27.8% (14.2-45.2%) and a higher specificity of 88.7% (83.3-92.6%). In total, based on current prescribing practices during the study, 57.8% of patients were correctly classified for the prescription of an antibiotic, while CRP-guided treatment would have increased this to 65.8% using a 20 mg/L threshold and to 79.3% using a 40 mg/L threshold.

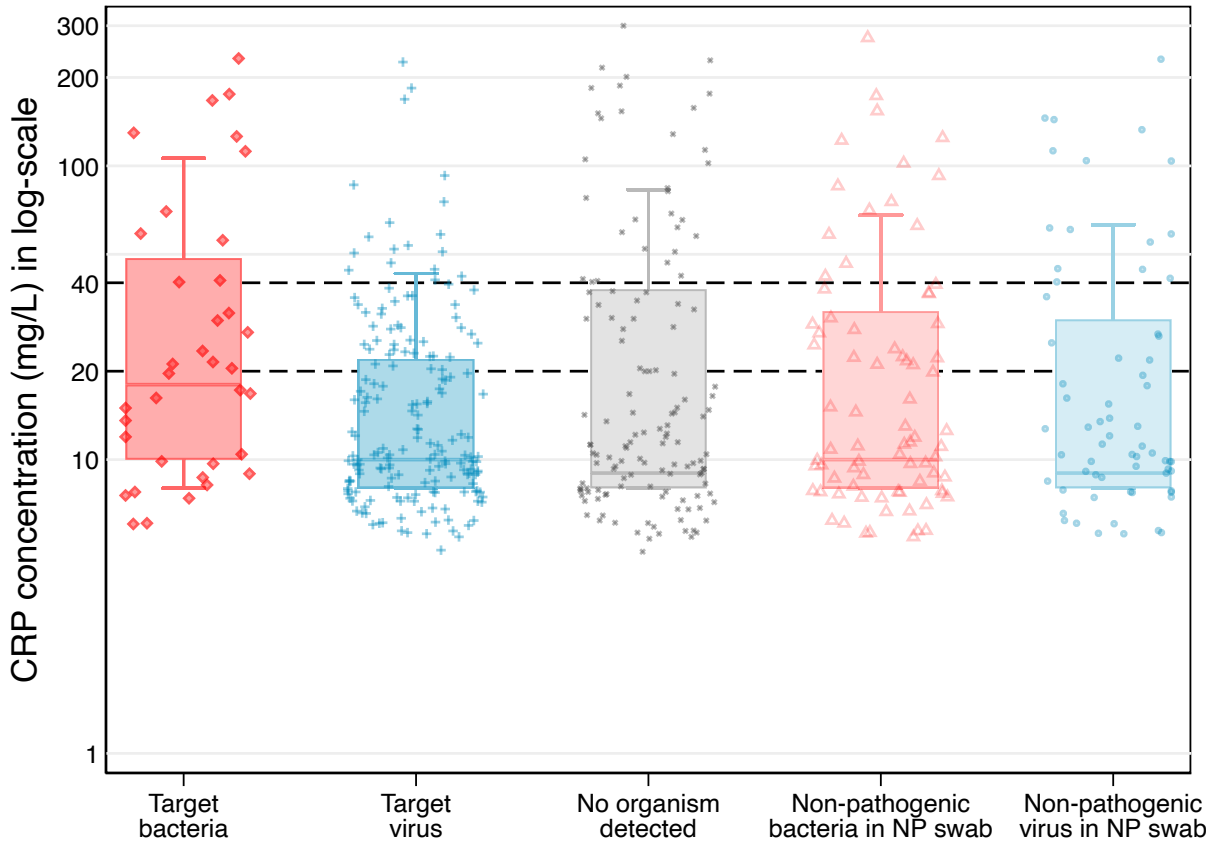


Figure 5-2 CRP concentration (mg/L) using a log scale per aetiological group in Chiang Rai, northern Thailand, and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

C-reactive protein (CRP) concentrations (all in log-scale) are coloured in dark blue for target viruses in blood and those detected in nasopharyngeal (NP) swabs with a high suspicion of causality.
Light blue colour corresponds to viruses detected in NP swabs with low evidence for causality.
Dark red colour corresponds to bacteria detected in blood and those detected in NP swabs with a high suspicion of causality.
Light red colour corresponds to bacteria detected in NP swabs with low evidence for causality.
Grey colour corresponds to patients for whom no organism was detected in blood specimens nor in NP swabs.
Each diamond, circle, cross, triangles or square corresponds to a measured CRP concentration.
Box boundaries shows 25th and 75th percentiles of CRP concentrations and line within the box shows the medians.
The whiskers indicate the 10th and 90th percentile of CRP concentrations.

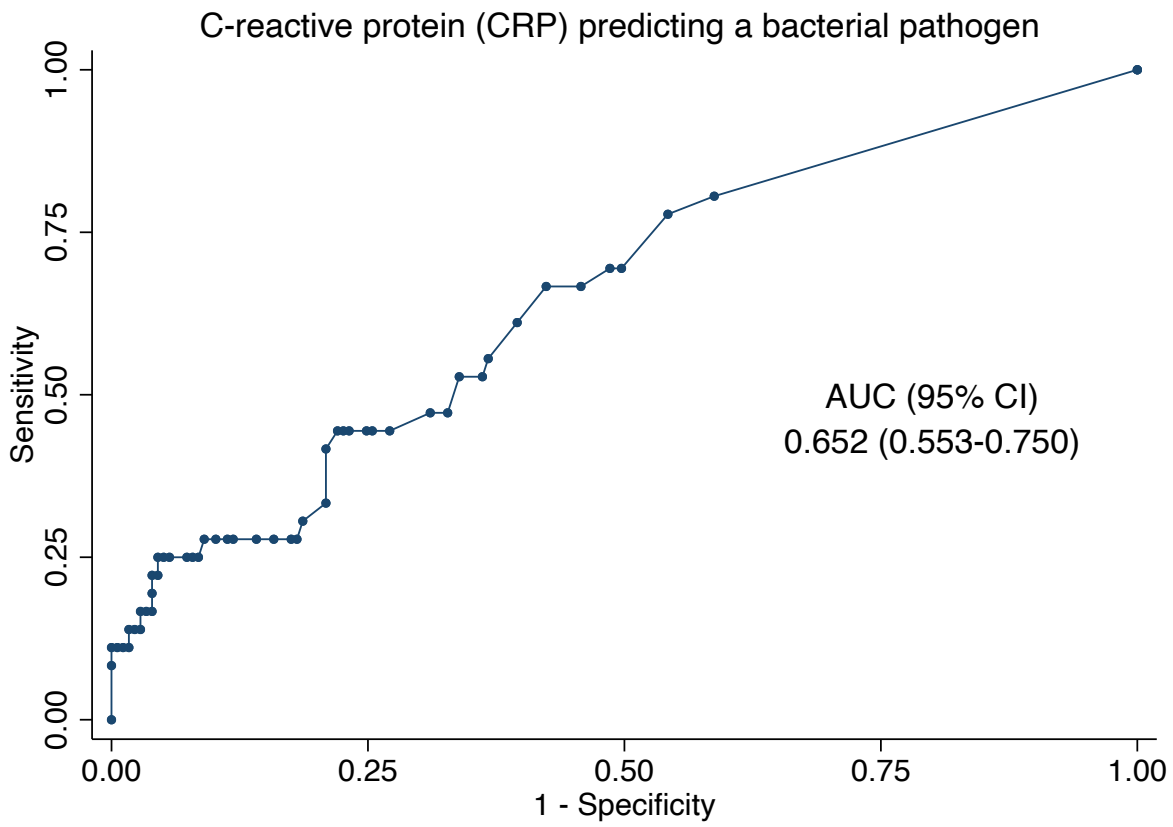


Figure 5-3 Diagnostic accuracy of CRP-testing for distinguishing between bacterial and viral pathogens in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017,

AUC – Area under the ROC curve
95% CI – 95% confidence interval

5.2.4 Patient outcomes by aetiological group

No evidence for a difference in antibiotic prescription was observed between the bacterial and viral groups at Day 0 (33.3% *versus* 39.8%, p-value 0.466, respectively), nor between Day 0 and Day 14 of the follow-up (38.9% *versus* 41.4%, p-value 0.782, respectively). Clinical outcomes were not significantly different between the two groups, as indicated in Table 5.4.

Among patients with a bacterial organism, two-thirds did not receive any antibiotic (24/36, 66.7%), and those antibiotics received were often ineffective for the detected bacteria. Out of 12 patients for whom either a macrolide or tetracycline (*Leptospira* spp. or *Rickettsia* genus) should have been prescribed, only one (8.3%) received the appropriate treatment. No difference in clinical outcomes was observed after 14 days of follow-up, regardless of whether an antibiotic was prescribed (p-values >0.05).

Table 5-4 Outcome characteristics by aetiological group in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Outcome characteristics	Bacteria (n=36)	Viruses (n=191)	p-value
Antibiotic prescription at Day 0, n (%)	12 (33.3)	76 (39.8)	0.466
- Broad-spectrum antibiotic at Day 0, n (%)	1/12 (8.3)	8/76 (10.5)	0.795
Antibiotic prescription from Day 0 - Day 14, n (%)	14 (38.9)	79 (41.4)	0.782
- Broad-spectrum antibiotic from Day 0 - Day 14, n (%)	2/14 (14.3)	8/79 (10.1)	0.621
Symptom resolution at Day 5, n (%)	23 (63.9)	115 (60.2)	0.519
Symptom severity at Day 5, median (IQR)	1 (1-1)	1 (1-1)	0.226
Documented fever at Day 5, n (%)	2 (5.6)	3 (1.6)	0.123
Elevated CRP at Day 5, n (%)	1 (2.8)	3 (1.6)	0.592
Symptom resolution at Day 14, n (%)	35 (97.2)	180 (94.2)	0.245
Symptom severity at Day 14, median (IQR)	1 (1-1)	1 (1-2)	0.103
Documented fever at Day 14, n (%)	0 (0)	1 (0.5)	0.665
Occurrence of SAE, n (%)	0 (0)	0 (0)	1.000
Unscheduled visits, n (%)	0 (0)	6 (3.1)	0.281
Hospitalisation, n (%)	0 (0)	(0)	1.000

Elevated C-reactive protein (CRP) at Day 5 was defined as ≥50 mg/L in children and ≥100 mg/L in adults
Severity was ranked from 1-4 with severity=1 being the less severe presentation
SAE: serious adverse event defined as events requiring hospitalisation or death within the 14 days of follow-up
Broad-spectrum antibiotics include ceftriaxone, cefixime, ciprofloxacin, levofloxacin, azithromycin, and amoxicillin with clavulanic acid.

5.3 Discussion

We investigated the spectrum of organisms among febrile children and adults in the community and evaluated the performance of CRP in distinguishing bacteria from viruses including its potential impact on antibiotic prescription compared with current practice. Patients were recruited prospectively across 10 sites in Thailand and Myanmar including urban, semi-urban and rural areas spanning over a full calendar year.

In our study, *Leptospira*, influenza, dengue and respiratory syncytial viruses were the leading organisms identified, which is consistent with previous reports in the region [21, 107]. The broad inclusion criteria, allowing for enrolment of all patients over 1 year old regardless of previous antibiotic intake, comorbidities, or clinical presentation, make our findings potentially more generalisable than previous studies. Investigating non-malarial febrile illness remains challenging in resource-limited areas [21], and despite the screening of multiple organisms on blood and respiratory specimens, we were only able to identify a probable cause of fever in 227 (29.4%) of patients. This low detection may be explained by the inclusion of only non-severe ambulatory patients [20, 363, 364], while other studies in Southeast Asia recruiting more severe and hospitalised patients identified an organism in around 50% of them [6, 7, 15, 110]. Only 15.9% (36/227) of organisms detected were bacteria, which may be explained by the lower risk of bacterial infections in non-severely ill patients, and where present, characterised by lower bacterial loads [364]. It has to be noted that the proportion of bacterial infections reported in hospital-based studies investigating causes of fever among more severe patients in LMICs does often not exceed 20% [7, 15, 23, 173].

Most bacteria were identified using a singleplex PCR and not the TAC assay, while viruses were equally detected by these two molecular methods. This lower sensitivity in the TAC assay for the detection of bacteria has been described in previous studies using multi-pathogen molecular detection platforms [365, 366]. The trade-off between advantages for screening multiple organisms at the same time with a simplified molecular platform should be weighed against potentially lower sensitivity, especially for bacteria such as *O. tsutsugamushi* or *Leptospira* spp., which are considered drivers of

febrile illness in Southeast Asia [57, 367]. Conversely the use of multiplex assays runs the risk of detecting organisms for which the causality and interpretation can be challenging: we detected *K. pneumoniae* among eight patients in blood, out of whom six did not receive any antibiotic. The detection of *K. pneumoniae* may be questionable, with its exclusion in a 2019 multi-country study because of poor assay specificity [368]. However, studies evaluating the TAC assay diagnostic accuracy for this bacterium found specificity over 98% using blood culture as a reference method [369, 370]. Regarding other bacteria detected in blood, it is challenging to know whether these should be considered a transient self-limiting infection or carriage. A 2018 community-based study also used the TAC assay to attribute a cause of bacterial infections among neonates in South Asia, but detected the presence of certain organisms among both controls and cases [62].

Furthermore, most of our patients presented with low CRP concentrations, regardless of whether a bacterial or viral organism was detected, and recovered regardless of whether an antibiotic was prescribed. It is likely that invasive bacterial infections requiring an antibiotic are exceptions while most primary care patients present with a self-limiting infection. Other primary care-based studies have recently supported restriction of antibiotic prescription to a small minority of patients: in Tanzania, a clinical trial lowered antibiotic prescription to 2.3% without affecting outcomes, while a U.S. study concluded that 72% of outpatients attending a general practice for a respiratory presentation should not even require a medical consultation let alone an antibiotic prescription [211, 242].

In our study, CRP performance in distinguishing bacterial from viral pathogens was average (AUC 0.65) and lower than another study in Southeast Asia (AUC 0.83 among 1,372 patients with a microbiologically-confirmed diagnosis [107]). It is likely that most bacteria detected in our study caused a self-limiting infection, while previous CRP evaluations included more severe and hospitalised patients with bacteraemia, detected by blood culture. While clearly of limited performance, CRP-guided treatment would still result in more accurate classification of fever as bacterial or viral aetiology compared with current practices (assuming that prescription of antibiotics was indicative of health workers believing the illness to be of bacterial origin). This supports the findings from the original trial where CRP-testing safely reduced antibiotic prescription, in line with similar primary care-based studies both in Asia and Africa [211, 274, 371].

Our study has several limitations: as mentioned above, the sensitivity of the multiplex TAC assay was not optimal for bacteria detection, and the absence of convalescent plasma impeded our ability to diagnose patients based on serology, particularly with respect to bacterial zoonoses. Blood culture was not available in this primary care-based study and this further limited our aetiological investigation. We did not recruit any concomitant control group, which precludes robust attribution of causality in the organisms we detected, particularly in NP swabs. In a paediatric study in Asia and Africa, the inclusion of controls matched with pneumonia cases weakened the evidence of causality for almost all organisms detected [368], and only RSV, hMPV, influenza virus A and B, parainfluenza virus type 1 and *B. pertussis* were found pathogenic, consistent with other LMIC-based studies [372, 373]. These organisms, however, are sometimes present in healthy controls, as shown in a literature review reporting the prevalence of influenza virus among healthy children at between 0-6%, RSV at 0-9% and hMPV between 0-7% [374]. On the other hand, we did not regard other organisms detected in NP swabs including rhinovirus, parainfluenza virus or *S. pneumoniae* as pathogenic, because these are commonly detected among healthy individuals [375, 376].

Chapter 6

THE CRP STUDY – SUSPICION OF EXPOSURE TO *BURKHOLDERIA PSEUDOMALLEI*

6 THE CRP STUDY – SUSPICION OF EXPOSURE TO *BURKHOLDERIA PSEUDOMALLEI*

6.1 Research in context

Burkholderia pseudomallei, the cause of melioidosis, is a saprophytic Gram-negative bacterium prevalent in tropical environments in moist soil and surface waters. The main modes of acquisition of melioidosis in both humans and animals are inoculation, inhalation and ingestion [377]. It is estimated that 165,000 cases of melioidosis occur globally every year, with about 40% of these in Southeast Asia where the mortality rate is 35% [344]. In North-eastern Thailand, more blood culture-confirmed cases of *B. pseudomallei* were reported in one year (2007) than the total combined number of cases from Singapore and Australia in a 10-year period, making this region a hotspot for melioidosis [378-382]. Studies from North-eastern Thailand consistently show high seroprevalence in humans [383, 384], and evidence of *B. pseudomallei* in the soil has also been demonstrated [385-387].

Seroprevalence data from other regions of Thailand is limited. Two surveys in northern Thailand reported 0.4-0.5% seropositivity in dairy cattle potentially infected with *B. pseudomallei* using the indirect hemagglutination assay (IHA) [388, 389]. An environmental study from this region in 1997 identified *B. pseudomallei* in 4.4% of soil samples [390]. Culture-confirmed melioidosis cases have been reported in northern Thailand from both Chiang Mai and Chiang Rai provinces [391, 392].

In neighbouring Myanmar data on melioidosis is scarcer despite the disease being initially described there with the first case ever reported identified in Rangoon (now Yangon), in 1911 [393]. This pathogen is currently not included in the Myanmar national surveillance system and has mainly been reported through sporadic case reports over the last 20 years [394]. A single hospital-based study of 3,865 blood cultures in Yangon did not identify any *B. pseudomallei* isolates between 2003-2015 [395]. In 2016, one seroprevalence survey using an enzyme-linked immunosorbent assay (ELISA) detecting antibodies against haemolysin-regulated protein 1 (Hcp1) and O-polysaccharide (OPS), reported 3.2% seropositivity for *B. pseudomallei* among 124 febrile adults from the delta region [396].

The current serological method, IHA, has shown poor diagnostic performance (sensitivity and specificity under 70% in North-eastern Thailand) [397]. ELISA assays used for detecting antibodies targeting *B. pseudomallei* antigens have been evaluated in culture-confirmed adults in both endemic and non-endemic areas, and outperformed IHA for the detection of *B. pseudomallei* [397, 398]. Antibodies against haemolysin co-regulated protein 1 (Hcp1) are raised in acute melioidosis and significantly decline after 52 weeks of follow-up. An anti-HCP1 IgG ELISA has shown potential for the sero-diagnosis of acute melioidosis (83.0% sensitivity and 96.3% specificity for detecting culture-confirmed infections in Northeast Thailand, with area under the receiver operating characteristic (ROC) curve of 0.95) [345].

To investigate human exposure to *B. pseudomallei* in Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, we performed a serological survey among febrile children and adults attending primary care using the anti-Hcp1 ELISA. We also compared the anti-Hcp1 ELISA between primary care febrile patients and healthy donors, matched by age category and geographical area in Chiang Rai, northern Thailand. This exposure is assumed by using a serodiagnostic method that has only been validated among culture-confirmed cases, while no study has yet validated anti-Hcp1 as a marker of seroprevalence: exposure to *B. pseudomallei* can therefore only be suspected and not ascertained.

6.2 Results

6.2.1 Patient characteristics

As shown in Table 6.1, children in Chiang Rai were younger than in Hlaing Tha Yar, while Chiang Rai adults were older than those from Hlaing Tha Yar (p-value <0.001). Agricultural activity was more frequent in Chiang Rai households than in Hlaing Tha Yar (p-value <0.001). Unemployment in households was higher in Hlaing Tha Yar than in Chiang Rai (p-value 0.008), and the proportion of skilled laborers was greater among Chiang Rai adults compared with those from Hlaing Tha Yar (p-value 0.002). Chiang Rai adults had less comorbidities than in Hlaing Tha Yar (p-value 0.029), but the proportion of diabetes was greater among Chiang Rai adults than those from Hlaing Tha Yar, although non-significantly (p-value 0.273).

Table 6-1 Demographic characteristics among febrile children and adults in Mueang Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

Total (n=656)	Children		p-value	Adults[a]		p-value
	Hlaing Tha Yar (n=101)	Chiang Rai (n=215)		Hlaing Tha Yar (n=164)	Chiang Rai (n=176)	
Female, % (n)	57.4 (58)	48.4 (104)	0.133	65.2 (107)	60.2 (106)	0.339
Age, median (IQR)	9 (7-13)	7 (4-9)	<0.001	32 (25-45)	49 (31-59)	<0.001
Professional level in the household, % (n)[b]						
- Agricultural activity	1.0 (1)	40.0 (86)	<0.001	1.2 (2)	36.4 (64)	<0.001
- No employment	5.9 (6)	0.9 (2)	0.008	18.3 (30)	8.5 (15)	0.008
- Non-skilled labourer	56.4 (57)	38.1 (82)	0.002	34.2 (56)	39.2 (69)	0.334
- Skilled labourer/professional	23.7 (24)	20.9 (45)	0.570	29.9 (49)	15.9 (28)	0.002
Low education level in the household, % (n)	62.5 (40)	75.8 (144)	0.290	41.3 (83)	47.3 (95)	0.099
Presence of any comorbidity, % (n)[c]	4.0 (4)	4.2 (9)	0.949	36.0 (59)	27.3 (48)	0.029
- Presence of diabetes, % (n)	0 (0)	0 (0)	1.000	3.7 (6)	6.3 (11)	0.273

[a]Adults defined as ≥18 years of age
[b]Skilled labourer included mechanic, factory worker, office, hotel or government employee; professional included doctor, teacher, lawyer or engineer; non-skilled labourer included housemaid, vendor or taxi driver
[c]Comorbidities included HIV, chronic hepatitis B or C, cirrhosis, diabetes mellitus, asthma, anaemia, chronic obstructive pulmonary disease, gastritis, congenital heart or kidney disease, alcoholism, dyslipidaemia, G6PD deficiency, hypertension, rheumatic heart disease, thalassaemia, thyroid disease.

6.2.2 Anti-Hcp1 antibody levels

In total, 656 febrile patients were screened for the anti-Hcp1 antibody using an Hcp1-ELISA. The median anti-Hcp1 IgG in Chiang Rai was significantly higher than in Hlaing Tha Yar (median optical density [IQR], 0.14 [0.05-0.69] *versus* 0.02 [0.01-0.03]; p-value <0.001), and this difference remained significant when analysing children and adults separately (p-value <0.001) as shown in Figure 6.1.

In Hlaing Tha Yar, anti-Hcp1 IgG levels were not different between children and adults (Table 6.2). However, in Chiang Rai, children had significantly higher levels than adults, including when analysing sites separately with the exception of one (Doi Hang, p-value 0.057). The OD median (IQR) decreased with age: 0.60 (0.17-1.19), 0.50 (0.11-1.02), 0.11 (0.04-0.38) and 0.06 (0.04-0.14) in patients under 7, 7-12, 13-18 and over 18 years old, respectively. Correspondingly, there was a significant reverse correlation between age and anti-Hcp1 levels, including when analysing sites separately in Chiang Rai (p-value <0.001).

In Hlaing Tha Yar, there were no differences in antibody levels between the four sites, including when analysing children and adults separately as shown in Table 6.2. On the other hand, there was a significant difference in antibody levels between the six sites in Chiang Rai (p-value <0.001). This difference remained when considering children and adults separately (p-value <0.001 and p-value 0.003 respectively). Children from Doi Lan and Huay Sak had the highest antibody levels (0.70 [0.29-1.54] and 0.70 [0.30-1.34], respectively), while the lowest levels were found among adults in Doi Hang and Mae Yao (0.10 [0.04-0.68] and 0.21 [0.06-0.69], respectively).

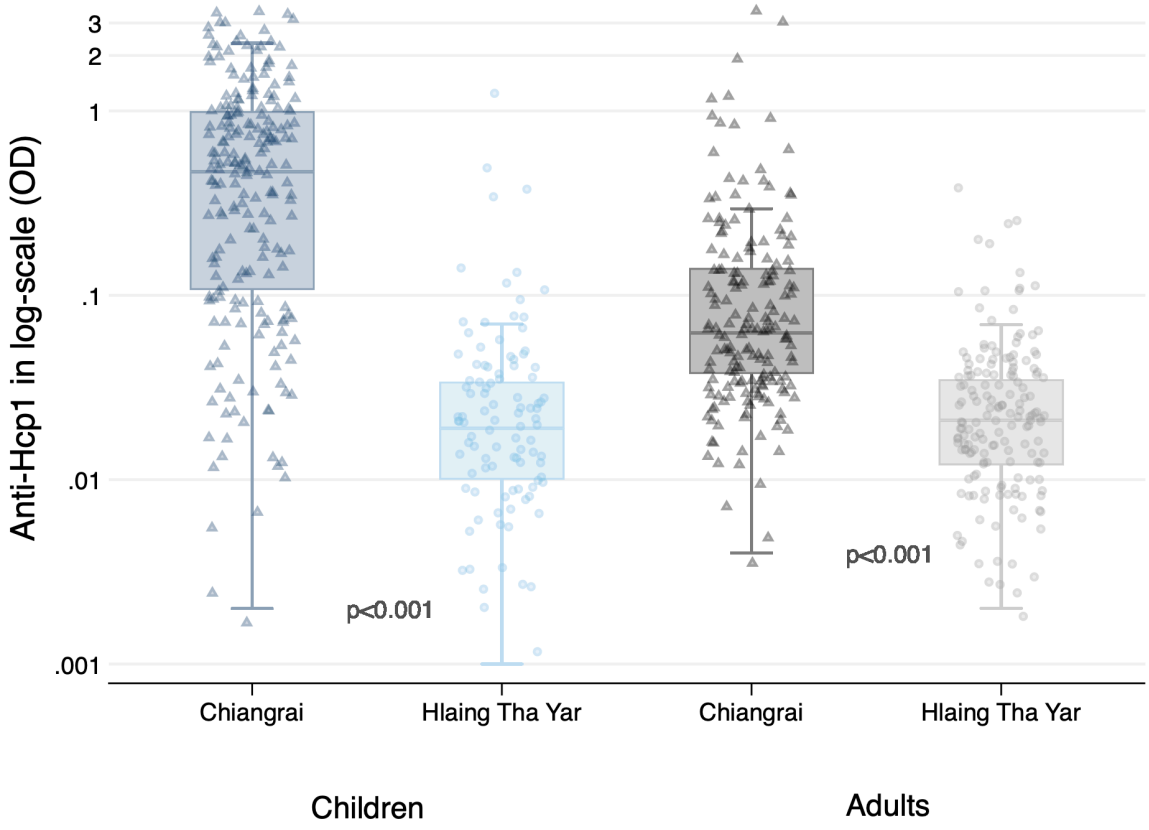


Figure 6-1 Anti-Hcp1 IgG levels in log-scale among febrile children and adults from Chiang Rai, northern Thailand, and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

The ELISA OD are shown in $\log_{10}$ scale.
Children are coloured in blue, while adults (defined as ≥18 years of age) are coloured in grey.
Patients from Chiang Rai and Hlaing Tha Yar are shown in triangles and circles, respectively.
Box boundaries shows 25th and 75th percentiles of the antibodies and line within the box shows the medians.
The whiskers indicate the 10th and 90th percentile of the antibodies

Table 6-2 Anti-Hcp1 IgG medians (IQR) in febrile children and adults in Mueang Chiang Rai, northern Thailand and Hlaing Tha Yar, Lower Myanmar, 2016-2017.

	Anti-Hcp1 IgG median (IQR)			
	Total	**Children**	**Adults[a]**	**p-value[b]**
Hlaing Tha Yar	**n=165**	**n=101**	**n=164**	
Four primary care units (n=265)	**0.02 (0.01-0.03)**	**0.02 (0.01-0.03)**	**0.02 (0.01-0.04)**	**0.507**
1. Hlaing Tha Yar A (n=82)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.192
2. Hlaing Tha Yar B (n=67)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.02 (0.01-0.03)	0.495
3. Hlaing Tha Yar OPD (n=31)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.03)	0.432
4. Shwepyithar (n=85)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.02 (0.01-0.04)	0.965
p-value[c]	0.903	0.584	0.807	
Mueang Chiang Rai	**n=391**	**n=215**	**n=176**	
Six primary care units (n=391)	**0.14 (0.05-0.69)**	**0.47 (0.11-1.00)**	**0.06 (0.04-0.14)**	**<0.001**
1. Doi Hang (n=50)	0.07 (0.05-0.36)	0.10 (0.04-0.68)	0.03 (0.02-0.09)	0.057
2. Mae Yao (n=73)	0.09 (0.04-0.31)	0.21 (0.06-0.69)	0.06 (0.04-0.10)	0.002
3. Ban Du (n=23)	0.12 (0.05-0.36)	0.59 (0.18-0.86)	0.09 (0.04-0.15)	0.022
4. Mae Khaow Tom (n=78)	0.16 (0.05-0.79)	0.46 (0.24-1.05)	0.05 (0.03-0.08)	<0.001
5. Huay Sak (n=65)	0.23 (0.07-0.72)	0.70 (0.30-1.34)	0.10 (0.05-0.20)	<0.001
6. Doi Lan (n=102)	0.30 (0.07-0.84)	0.70 (0.29-1.54)	0.09 (0.04-0.20)	<0.001
p-value[c]	<0.001	<0.001	0.003	

[a]Patients with age over 18 years old are defined as adults
[b]p-value measured for anti-Hcp1 IgG difference between children and adults for a given study site
[c]p-value measured for anti-Hcp1 IgG difference between study site for a given age category

Geographical locations of each primary care site in Chiang Rai with corresponding medians of anti-Hcp1 IgG levels are illustrated in Figure 6.2.

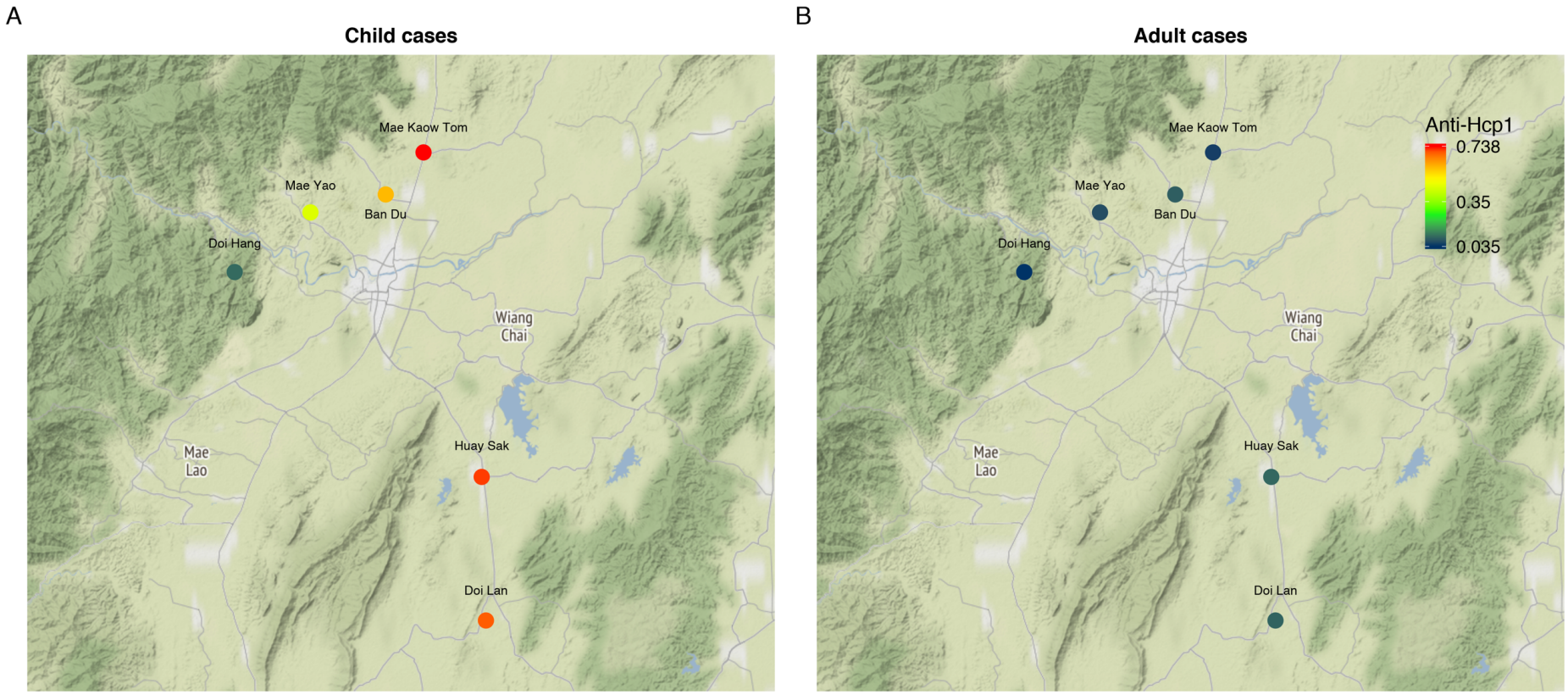


Figure 6-2 Primary care sites by age category in Chiang Rai, northern Thailand, 2016-2017.

Each dot represents the median of anti-Hcp1 IgG among febrile children (panel A) and adults (defined as ≥18 years of age, panel B). The anti-Hcp1 IgG (median optic density) are shown in a gradient colour

6.2.3 Anti-Hcp1 antibody among healthy donors in Mueang Chiang Rai

To investigate anti-Hcp1 IgG in the non-febrile population of Mueang Chiang Rai, a total of 74 healthy donors (39 children, 35 adults) from 10 subdistricts were tested using the anti-Hcp1 ELISA (Table 6.3). Anti-Hcp1 IgG levels were significantly higher in children than adults (p-value <0.001), but this difference became not significant when considering each subdistrict independently. There was a significant difference in antibody levels between the 10 subdistricts (p-value 0.018), although this difference was not significant when analysing children and adults separately.

Table 6-3 Anti-Hcp1 IgG medians (IQR) in healthy children and adults in ten subdistricts of Mueang Chiang Rai, northern Thailand, 2015-2016.

	Anti-Hcp1 IgG median (IQR)			
	Total (n=74)	**Children (n=39)**	**Adults[a] (n=35)**	**p-value[b]**
Mueang Chiang Rai				
Ten subdistricts (n=74)	**0.03 (0.02-0.09)**	**0.07 (0.03-0.19)**	**0.03 (0.02-0.04)**	**<0.001**
1. Mae Yao (n=24)	0.04 (0.03-0.13)	0.08 (0.03-0.19)	0.03 (0.02-0.04)	0.096
2. Doi Hang (n=17)	0.07 (0.04-0.23)	0.12 (0.04-0.31)	0.06 (0.04-0.13)	0.428
3. Huay Chompu (n=13)	0.03 (0.01-0.03)	0.01 (0.01-0.03)	0.03 (0.02-0.05)	0.341
4. Ban Du (n=3)	0.02 (0.01-0.03)	X	0.02 (0.01-0.03)	X
5. Mae Salong Nai (n=3)	0.02 (0.01-0.06)	0.06 (0.06-0.06)	0.01 (0.01-0.02)	0.221
6. Mae Salong Nok (n=2)	0.06 (0.03-0.09)	0.09 (0.09-0.09)	0.03 (0.03-0.03)	0.317
7. Pa Daet (n=2)	0.03 (0.03-0.04)	X	0.03 (0.03-0.04)	X
8. Rim Kok (n=3)	0.02 (0.02-0.10)	0.10 (0.10-0.10)	0.02 (0.02-0.02)	0.157
9. Tha Ko (n=4)	0.02 (0.02-0.03)	X	0.02 (0.02-0.03)	X
10. Wawi (n=3)	0.05 (0.03-0.09)	X	0.05 (0.03-0.09)	X
p-value[c]	0.018	0.301	0.120	

[a]Patients with age over 18 years old are defined as adults
[b]p-value measured for anti-Hcp1 IgG difference between children and adults for a given subdistrict
[c]p-value measured for anti-Hcp1 IgG difference between subdistricts overall and for a given age category

Out these 10 Chiang Rai subdistricts where healthy donors were sampled, two were common with the CRP Study: Doi Hang and Mae Yao. Considering those two subdistricts, we counted 41 healthy donors (19 children, 22 adults) and 123 febrile primary care patients (67 children, 56 adults). Both healthy and febrile children living in these two subdistricts had similar antibody medians (0.09 [0.03-0.23] and 0.14 [0.04-0.69], p-value 0.360, respectively). In the same way, both healthy and febrile adults from these two subdistricts had similar antibody medians (0.03 [0.02-0.05] and (0.05 [0.03-0.09], p-value 0.065, respectively), as illustrated in Figure 6.3.

When comparing febrile patients from Lower Myanmar with healthy donors from northern Thailand, the latter had significantly higher antibody levels, including when analysing children and adults separately (p-value <0.001).

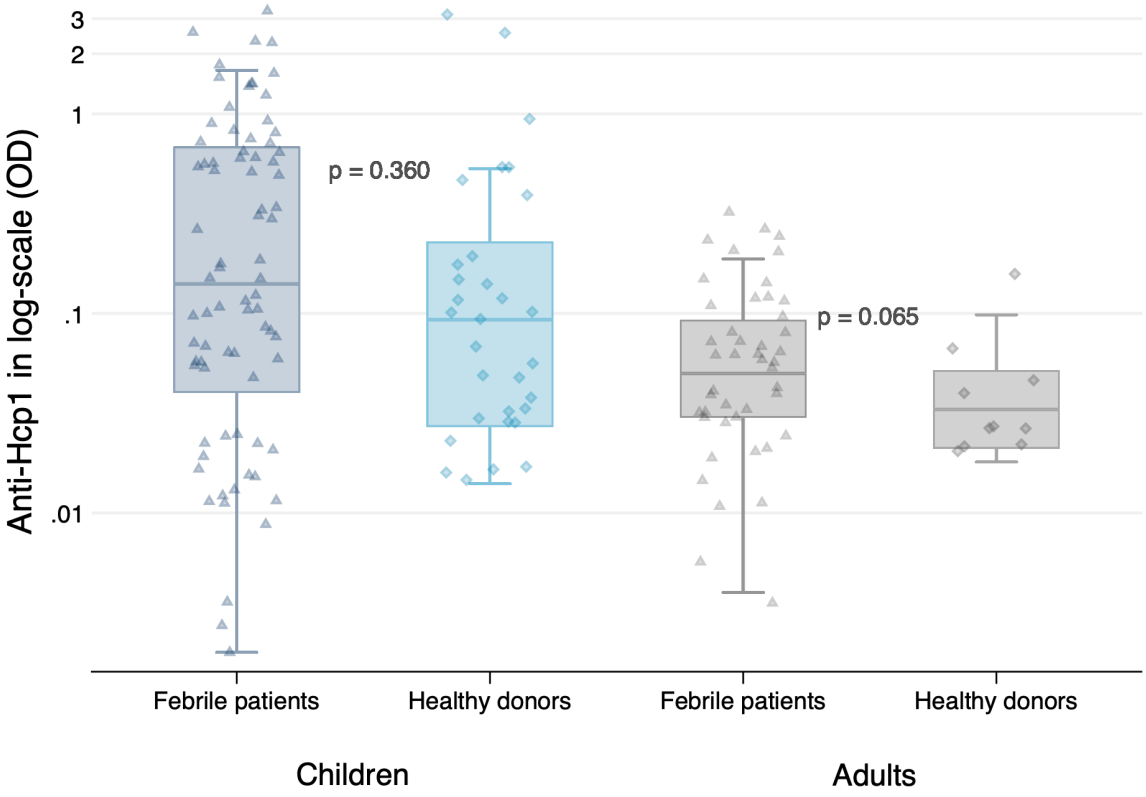


Figure 6-3 Anti-Hcp1 IgG levels in log-scale among febrile patients (2016-2017) and healthy donors (2015-2016) matched by age category and subdistrict in Mueang Chiang Rai, northern Thailand.

The ELISA OD are shown in $\log_{10}$ scale.
Children are coloured in blue while adults (defined as ≥18 years of age) are coloured in grey.
Febrile patients and healthy donors are shown in triangles and diamonds, respectively.
Box boundaries shows 25th and 75th percentiles of the antibodies and line within the box shows the medians.
The whiskers indicate the 10th and 90th percentile of the antibodies.

6.3 Discussion

6.3.1 Age and geographical heterogeneity

Anti-Hcp1 IgG levels were elevated among northern Thai febrile children attending primary care, which may be related to a recent exposure to *B. pseudomallei*. To date, data estimates of the burden of *B. pseudomallei* have been predominantly drawn from studies in adults and hospital settings with limited investigations based in the community and in children [399, 400].

Seroprevalence studies have mainly been conducted using IHA, but its low sensitivity and specificity make previous results of the exposure to *B. pseudomallei* unreliable [401-403]. The anti-Hcp1 ELISA has been demonstrated to outperform the IHA for the identification of *B. pseudomallei* among culture-confirmed adults in both endemic and non-endemic regions, as Hcp1 is immunogenic and structurally different from non-pathogenic *Burkholderia* species such as *B. thailandensis* [345, 404]. Previous evaluations of the Hcp1-ELISA showed high sensitivity and specificity in detecting melioidosis patients with controls including healthy adults originated from endemic area (North-eastern Thailand) and non-endemic area (United States of America): the detection sensitivity ranged from 83 to 94%, and the detection specificity between 96 and 100%, when using a cut-off OD of 1.165 [345, 405].

The youngest febrile patients (under 7-year-old) had the highest antibody levels against *B. pseudomallei* in Mueang Chiang Rai, which was also the case among under 7-year-old healthy donors when comparing with those over 18 years of age (p-value 0.008, data not shown). This is consistent with previous reports describing the greatest seroconversion rate (based on IHA titres) during the first years of life among children living in endemic areas in Northeast Thailand [383, 384, 406]. However, this was not the case among febrile children from Hlaing Tha Yar, Lower Myanmar. This suggests some differences in the microbial exposome between these two countries including different lifestyles, dietary components and water supply systems [407, 408]. The child immune development is influenced by the composition of the maternal microbiome, through mucosal contacts during pregnancy, and by environmental exposure after birth. The youngest children are defined by an immature immune system,

and factors such as exposures through minor skin abrasions and ingestion of contaminated water might explain why we and previous studies measured such high IHA titres or anti-Hcp1 ELISA OD during the first years of life and not among adults [384, 409, 410].

IHA titres usually persist over years [384, 411], while anti-Hcp1 IgG antibody levels significantly decline within 52 weeks (following acute melioidosis) [345]. This suggests that anti-Hcp1 IgG may represent a better marker of recent exposure to *B. pseudomallei* than the IHA titre.

Geographical heterogeneity of *B. pseudomallei* distribution has been described within a single region, which is consistent with the marked differences in antibody levels found across the six Mueang Chiang Rai sites and the ten Mueang Chiang Rai subdistricts [386, 412]. Factors usually associated with the presence of *B. pseudomallei* include soil type and water turbidity. Farming is also a well-described risk factor we identified in our study [344]. Endemic areas with high seroprevalence of *B. pseudomallei* among humans commonly present corresponding microbiological evidences in the environment [387, 413, 414], especially in Northeast Thailand [383-386]. This suggests the necessity to sample soil in Mueang Chiang Rai region, especially in the subdistricts where we measured the highest levels of anti-Hcp1.

In Hlaing Tha Yar, located in the west of Yangon, we observed low antibody levels. This is consistent with the literature where *B. pseudomallei* was neither isolated from the 3,865 blood cultures between 2003 and 2015 in Yangon, nor from environmental sampling in this urban environment [395, 415]. However, in the rural delta region of Myanmar, 3.2% of febrile adults were recently identified as seropositive based on ELISA, with environmental evidence of *B. pseudomallei* confirmed by molecular methods throughout the country, suggesting that melioidosis should be included in a national surveillance programme including rural areas in Myanmar [394, 396].

In our analysis, we did not apply a serodiagnosis cut-off previously used to evaluate the Hcp1-ELISA [345]. If a cut-off of OD >1.165 (determined by using a specificity of 95% using Thai healthy adults as controls) was applied, none of the 265 patients from Hlaing Tha Yar would have been seropositive, while 13.0% (51/391) of the febrile patients in Chiang Rai would be considered seropositive (47 children (21.9%) and 4 adults (2.3%)). It is therefore possible that some of our febrile

patients may have a subclinical infection [416]. Samples with anti-Hcp1 IgG levels of OD >1.165 also had consistently elevated anti-OPS antibodies using an anti-OPS IgG ELISA (Table and Figure 6.4).

Table 6-4 Correlation between anti-Hcp1 and anti-OPS IgG levels among overall febrile patients, children and adults with an anti-Hcp1 IgG OD >1.165 in Mueang Chiang Rai, northern Thailand, 2016-2017.

Correlation between anti-Hcp1 & OPS IgG	Pearson's coefficient	p-value
Overall (n=51)	0.92	<0.001
Children (n=47)	0.98	<0.001
Adults[a] (n=4)	0.95	0.050

[a]Patients with age over 18 years old are defined as adults
OPS is O-polysaccharide
Hcp1 is haemolysin co-regulated protein 1

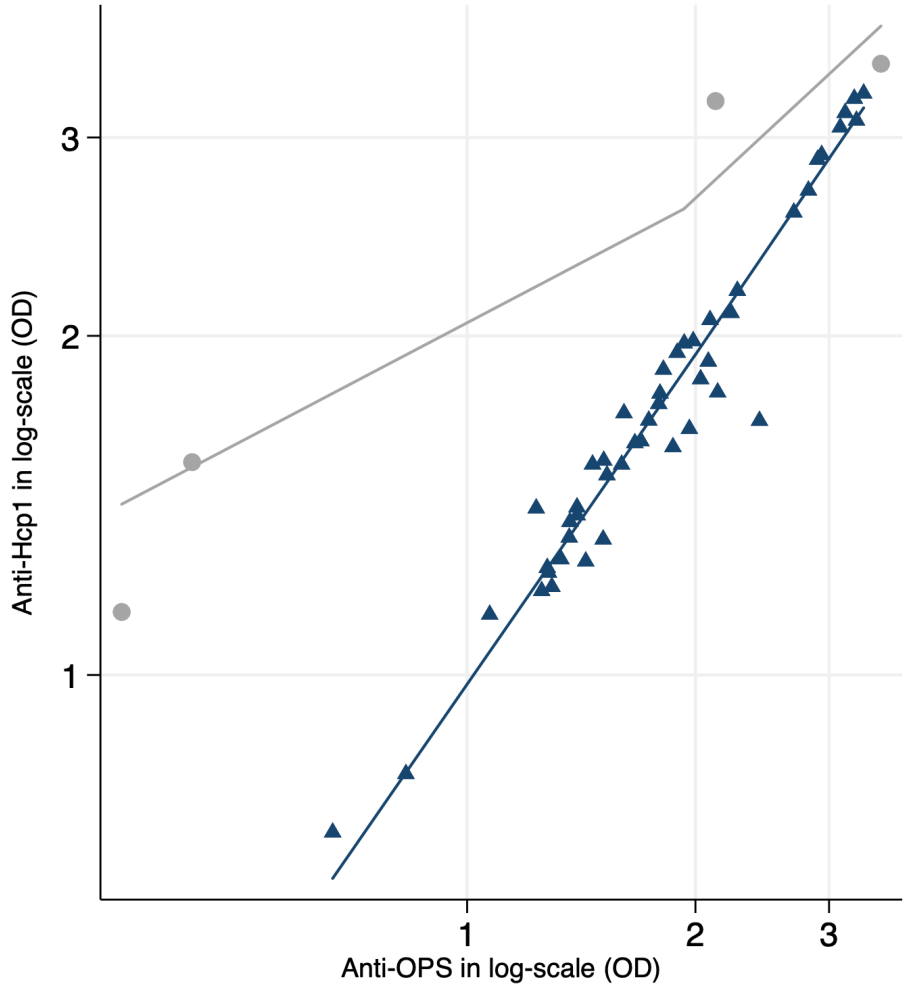


Figure 6-4 Anti-Hcp1 and anti-OPS IgG levels (in log scale) among febrile children and adults for Hcp1-ELISA OD >1.165 in Mueang Chiang Rai, northern Thailand, 2016-2017.

Children are coloured in blue triangles and adults (defined as ≥18 years of age) in grey circles.
OD is optical density
OPS is O-polysaccharide
Hcp1 is haemolysin co-regulated protein 1

6.3.2 Limitations

The anti-Hcp1 IgG antibody has only been validated among culture-confirmed melioidosis adults and not among children [345, 405]. During this validation, the anti-Hcp1 median OD was 0.17 (0.07-0.38) among U.S. healthy donors used as controls, which is biologically similar to the median levels measured in our febrile adults in Chiang Rai (0.06 [0.04-0.14]). This would either suggest that Chiang Rai febrile adults have not recently been exposed to *B. pseudomallei*, or that adults only express antibody against Hcp1 during an invasive and active melioidosis infection, and not on environmental exposure to *B. pseudomallei*. In addition, this validation did not include paediatric melioidosis culture-confirmed cases: whether the high Hcp1-ELISA measured among children (0.47 [0.11-1.00]) is linked to an exposure to *B. pseudomallei* remains unclear.

Another limitation deals with the use of healthy donors as controls. These healthy donors were very few (19 children, 22 adults), sampled prior to the febrile patients (2015-2016 *versus* 2016-2017), matched by subdistricts rather than by exact addresses, and were only from subdistricts where antibody levels were the lowest in the CRP study. Although similarly low antibody levels between healthy donors and febrile patients may suggest a consistent exposure in Mueang Chiang Rai, the absence of data among healthy donors in subdistricts where febrile patients showed the highest antibody levels limits the verification of this assumption.

By contrast to IHA, which defines various cut-offs for quantifying exposure, no Hcp1-ELISA cut-off for exposure exists, only for serodiagnosis [345]. It was therefore impossible to prove an exposure or measure the seroprevalence among individuals exposed to *B. pseudomallei*, and the meaning of such exposure on long-term clinical outcome remains unknown. Longitudinal studies including culture-confirmed paediatric samples with matched healthy children from both endemic and non-endemic are warranted.

Chapter 7

THE TANZANIAN STUDY – SENSITIVITY OF C-REACTIVE PROTEIN IN IDENTIFYING BACTERIAL PATHOGENS

7 THE TANZANIAN STUDY – SENSITIVITY OF C-REACTIVE PROTEIN IN IDENTIFYING BACTERIAL PATHOGENS

7.1 Research in context

With 92% of malaria cases and 93% of malaria deaths in the world, Africa bears a far greater burden than Southeast Asia, however, a significant reduction of *Plasmodium falciparum* as a cause of fever has been reported in the past decade [120, 135]. In Tanzania for instance, a 2013 study found that out of two-thirds of clinically suspected malaria cases, only 1.6% were confirmed, whereas bacterial zoonosis was the most common aetiology at 26.2% [121]. In 2014, a Tanzanian primary care-based study identified *P. falciparum* in only 10.5% of febrile children, while viruses were the most frequent pathogens detected [23].

The rarefaction of acute fevers caused by malaria in sub-Saharan Africa poses the question of how to manage febrile patients, particularly in settings where laboratory capacity is limited [417, 418]. The management of malaria has improved by the use of malaria rapid diagnostic tests, reducing the unnecessary use of antimalarials [419]. However, health workers lack tools to identify other causes of fever once the malaria test is negative, which favours the irrational use of antibiotics and worsens the spread of antibiotic resistance [15, 121, 420]. Primary care is the setting where access to diagnostics is the most limited, and antibiotic prescription is consequently high: in Nigeria, for instance, prescriptions reached 85% among under-5 children with diarrhoea although 90% of them had rotavirus detected in the stools, and more than two thirds of South African children and adults presenting with an acute condition received at least one antibiotic in 2016-2017 [255, 421]. Antibiotic overuse extends to higher facility levels: 79% of 3,654 Eritrean inpatients from a national referral hospital were prescribed an antibiotic irrespective of the diagnosis, similar to examples found in Ethiopia (73.7%), Sudan (81.3%)

and Democratic Republic of Congo (DRC) (68%) [422-425]. Prescription levels were even higher in Tanzania, where 84.9% of children with respiratory or digestive symptoms received an antibiotic [426].

Host-response biomarkers discriminating bacterial from viral pathogens have been evaluated as a potential tool to guide health workers' antibiotic prescription, but evidence for their utility in low- to middle-income countries (LMICs) is scant, particularly in Africa [233, 361]. In Southeast Asia, C-reactive protein (CRP) has shown a high sensitivity and moderate specificity for differentiating between bacterial and viral pathogens, both at primary care and hospital levels, and including among children and adults with various clinical presentations [313, 316, 427]. In Africa however, most studies of CRP performance are limited to hospitalised patients presenting with a severe respiratory tract infection [129, 130, 428, 429]. A 2019 study focused on patients with a neurological disorder and demonstrated high performance of CRP for ruling out invasive bacterial infections in a rural hospital in DRC [430]. Furthermore, CRP evaluations rarely include zoonotic pathogens, while sub-Saharan Africa is considered to bear a high burden for zoonoses [431].

We therefore aimed to measure the CRP concentration among febrile children and adults attending both hospital and primary healthcare settings regardless of clinical presentation, with culture-confirmed bacterial bloodstream infections (BSI) and bacterial zoonotic infections. We also calculated the sensitivity of CRP in detecting these bacterial infections as requiring antibiotic treatment, evaluating 10, 20, and 40mg/L thresholds, based on the literature [110, 316, 430, 432]. This prospective observational study was implemented in Moshi, northern Tanzania, between 2011-2014.

7.2 Results

7.2.1 Patient characteristics

A total of 804 out of 1,753 febrile patients (45.9%) included between September 2011 and May 2014 had sufficient sample volume to be tested for CRP. Patient characteristics are presented in Table 7.1. Of 804, 235 (29.2%) were outpatients and 569 (70.8%) were inpatients. Outpatients were significantly younger and reported a lower HIV prevalence, shorter duration of symptoms and lower antimalarial consumption prior to enrolment compared with inpatients (p-value <0.05). Within outpatients, children (defined as age ≤10-year-old) accounted for 16.2% (38/235) and 2.3% (13/569) among inpatients.

Clinically, outpatients tend to be more febrile and less likely to present with gastrointestinal symptoms than inpatients (p-value <0.05). The most frequent clinical presentation among outpatients was neurological symptoms (69.4%), followed by gastrointestinal and respiratory symptoms (68.9% and 68.1%, respectively). Among inpatients, gastrointestinal symptoms were the most common (79.4%), followed by neurological and respiratory symptoms (74.7% and 69.2%, respectively). There were no significant differences in neurological and respiratory presentations between outpatients and inpatients (p-values 1.131 and 0.773, respectively). Febrile patients presenting without any clinical focus represented the less frequent presentation among both outpatients and inpatients (p-value 0.101).

Table 7-1 Demographic and clinical characteristics of febrile outpatients and inpatients, Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

Enrolment characteristics	Outpatients (n=235)		Inpatients (n=569)	
	Children (n=38)	Adults (n=197)	Children (n=13)	Adults (n=556)
Demographic characteristics				
Age in years, median (IQR)	6 (4-7)	34 (24-42)	4 (2-5)	36 (27-45)
Self-reported HIV infection, n (%)	1 (2.6)	13 (6.6)	1 (7.7)	133 (23.9)
Other healthcare facility prior to enrollment, n (%)	22 (57.9)	127 (64.5)	8 (61.5)	346 (62.1)
Fever duration in days, median (IQR)	3 (2-4)	5 (3-12)	3 (2-3)	5 (3-14)
Antibacterial intake prior to enrollment, n (%)	5 (13.6)	82 (41.6)	7 (53.9)	227 (40.8)
Antimalarial intake prior to enrollment, n (%)	6 (15.8)	57 (28.9)	5 (38.5)	200 (35.9)
Clinical characteristics				
Tympanic temperature (˚C), median (IQR)	38.9 (38.3-39.3)	38.4 (38.1-38.9)	38.1 (37.5-39.0)	38.1 (37.4 -38.7)
Neurological symptoms, n (%)	23 (60.5)	140 (71.1)	5 (38.5)	420 (75.4)
Respiratory symptoms, n (%)	29 (76.3)	131 (66.5)	11 (84.6)	383 (68.8)
Gastro-intestinal symptoms, n (%)	24 (63.2)	138 (70.1)	10 (76.9)	442 (79.4)
Undifferentiated presentation, n (%)	4 (10.5)	4 (2.0)	0 (0)	9 (1.6)

Children were defined as age ≤10-year-old
Antibiotic and antimalarial intake prior to enrolment was based upon patient or caregiver report.
Neurological symptoms include headache, convulsions, or stiff neck.
Respiratory symptoms include sore throat, dyspnoea, haemoptysis, or cough.
Gastrointestinal symptoms include nausea, vomiting, diarrhoea, constipation, jaundice, bloody stools, or abdominal pain.
Undifferentiated presentation defined by the absence of any symptoms present in neurological, respiratory, nor gastrointestinal systems. Common symptoms in this group included myalgia, arthralgia, tiredness, chills, sweating, rash, bleeding, or appetite loss.

When comparing patients with and without sufficient sample volume for CRP-testing by age category, children included in the CRP evaluation tended to be older with less antibiotic intake prior to enrolment, and are more likely to present with neurological symptoms compared with children who did not have sufficient sample left (p-value <0.05). Adults with sufficient sample for CRP-testing were significantly older, with a higher duration of symptom onset and more frequent neurological presentation compared with adults who did not have sufficient sample left (p-value <0.05). Comparison of these two groups are detailed in Table 7.2.

Table 7-2 Comparison of demographic and clinical characteristics between patients with and without CRP-testing by age category, Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

Enrolment characteristics	Children (n=695)			Adults (n=1,058)		
	No CRP-testing (n=644)	CRP-testing (n=51)	p-value	No CRP-testing (n=305)	CRP-testing (n=753)	p-value
Demographic characteristics						
Age in years, median (IQR)	1 (0-3)	5 (3-7)	<0.001	34 (23-44)	36 (26-45)	0.010
Self-reported HIV infection, n (%)	6 (0.9)	2 (3.9)	0.146	52 (17.1)	146 (19.4)	0.926
Other healthcare facility prior to enrolment, n (%)	396 (61.5)	30 (58.8)	0.472	170 (55.7)	473 (62.8)	0.510
Fever duration in days, median (IQR)	3 (2-5)	3 (2-4)	0.114	4 (3-10)	5 (3-14)	0.048
Antibiotic intake prior to enrolment, n (%)	240 (37.3)	12 (23.5)	0.049	124 (40.7)	309 (41.0)	0.909
Antimalarial intake prior to enrolment, n (%)	145 (22.5)	11 (21.6)	0.876	87 (28.5)	257 (34.1)	0.078
Clinical characteristics						
Tympanic temperature (˚C), median (IQR)	38.4 (38.1-39.1)	38.7 (38.1-39.3)	0.091	38.3 (38.0-38.8)	38.2 (37.6-38.8)	0.058
Neurological symptoms, n (%)	135 (21.0)	28 (54.9)	<0.001	168 (55.1)	560 (74.4)	<0.001
Respiratory symptoms, n (%)	470 (73.0)	40 (78.4)	0.397	211 (69.2)	514 (68.3)	0.770
Gastro-intestinal symptoms, n (%)	476 (73.9)	34 (66.7)	0.260	242 (79.3)	580 (77.0)	0.412
Undifferentiated presentation, n (%)	32 (5.0)	4 (7.8)	0.373	5 (1.6)	13 (1.7)	0.921

7.2.2 Antibiotic prescription

The median (IQR) CRP value for all patients was 75 (18-176) mg/L, which was lower among outpatients than inpatients (46 mg/L [9-119] and 93 mg/L [31-199], respectively, p-value <0.001). After enrolment and medical examination by the health worker, 174 (74.0%) outpatients and 487 (85.6%) inpatients were prescribed an antibiotic (p-value <0.001). Prescription by CRP concentration is illustrated in Figure 7.1, including broad-spectrum antibiotics. Among 174 outpatients who had an antibiotic prescribed, 95 (54.6%) were broad-spectrum, while there were 317 out 487 (65.1%) among inpatients, and the prescription of broad-spectrum antibiotics did not vary across CRP concentrations (p-value 0.584).

Considering the 51 children included in our study, 32 out 38 (84.2%) outpatients received an antibiotic, while all of the 13 inpatients had an antibiotic prescribed. Broad-spectrum antibiotics were prescribed in 19 of 38 (59.4%) and 5 of 13 (38.5%) among paediatric outpatients and inpatients, respectively.

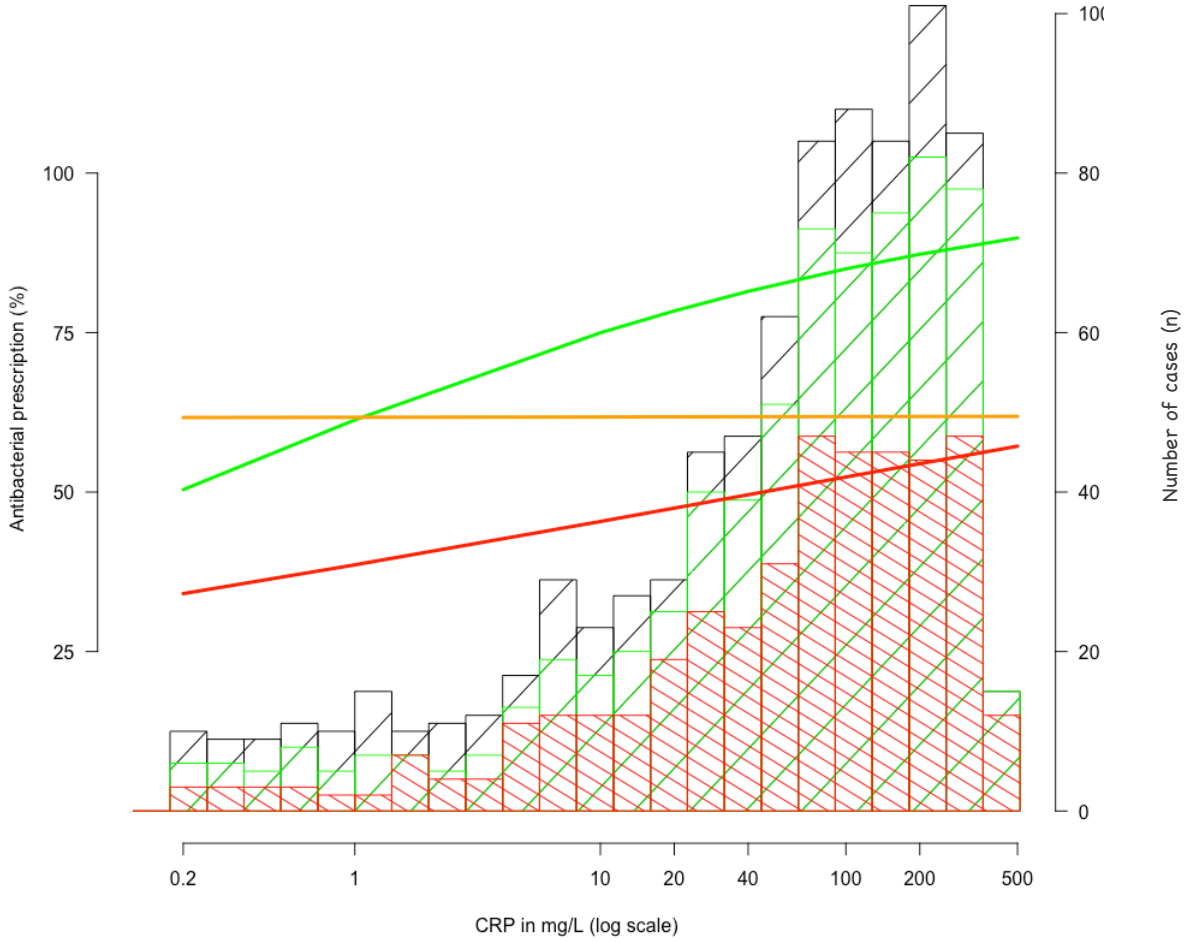


Figure 7-1 Antibiotic prescription among febrile outpatients and inpatients by CRP concentrations (log-scale), Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

Overall and broad-spectrum antibacterial prescription (green and red respectively) overlaid with all patients (black) across CRP distributions (log scale).
The lines represent the logistic regression model between CRP levels and: i. Prescription of overall antibacterials (green); ii. Prescription of broad-spectrum antibacterials (red); iii. Prescription of broad-spectrum antibacterials among those with an antibacterial (orange).

Considering patients with a CRP <10 mg/L, 37 of 60 (61.7%) outpatients and 52 of 77 (67.5%) inpatients had an antibiotic prescribed; of these, 22 (59.5%) and 33 (63.5%), respectively, received a broad-spectrum antibiotic. Among children, 9 out 13 (69.2%) outpatients and all of the thirteen inpatients had an antibiotic prescribed for CRP concentrations below 10 mg/L.

Considering all 234 outpatients, 190 (80.9%) of 235 were discharged, 41 (17.5%) were transferred to the hospital ward, and we did not find outcomes for the four remaining outpatients. Considering 569 inpatients, 525 (92.3%) were discharged, 30 (5.3%) were transferred to other facilities, and 15 (2.6%) died in hospital. Among the 15 inpatients who died, all of them were adults, median CRP was 85 (39-150) mg/L, and all of them were prescribed an antibiotic, including a broad-spectrum in 12 out 15 (80.0%) cases. Two inpatient deaths presented with CRP concentrations below 10mg/L: one had a final diagnosis of documented cryptococcal meningitis and the other one was diagnosed with a nephrotic syndrome.

7.2.3 Microbiologically-confirmed diagnosis

Out of 804 patients included, 107 (13.3%) had a microbiologically-confirmed diagnosis assigned, including 34 BSIs (31 bacterial and 3 fungal), 61 bacterial zoonoses, and 12 *P. falciparum* cases; co-infections were detected in five patients as detailed in Table 7.3.

Table 7-3 Pathogens detected and aetiological groupings among febrile outpatients and outpatients by age category, Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

Pathogen	Outpatients		Inpatients	
	Children	Adults	Children	Adults
Bacterial BSI (n=31)	**n=2**	**n=6**	**n=1**	**n=22**
E. coli	0	2	0	11
E. coli + *Brucella*	0	1	0	0
Enterobacteriaceae	0	0	0	2
K. pneumonia + *Leptospira*	0	0	0	1
Non-typhoidal *Salmonella*	0	0	0	2
Pseudomonas + *P. falciparum*	0	0	0	1
S. pneumoniae	1	0	0	0
S. pneumoniae + *Brucella**	0	0	0	1
S. pyogenes	0	0	0	1
S. enterica serovar Typhi	1	3	1	3
Bacterial zoonosis (n=61)	**n=0**	**n=17**	**n=2**	**n=42**
Brucella	0	8	1	19
Brucella + *P. falciparum*	0	0	0	1
Brucella + *R. africae*	0	0	0	1
C. burnetii	0	0	1	3
C. burnetii + *R. africae*	0	0	0	1
Leptospira	0	6	0	9
Leptospira + *Brucella*	0	2	0	1
Leptospira + *C. burnetii*	0	0	0	1
R. africae	0	1	0	6
Fungal BSI (n=3)	**n=0**	**n=0**	**n=0**	**n=3**
C. neoformans	0	0	0	3
Malaria (n=12)	**n=0**	**n=2**	**n=0**	**n=10**
P. falciparum	0	2	0	10
Total (n=107)	**n=2**	**n=25**	**n=3**	**n=77**

BSI – Blood stream infection

All detections of *Brucella* were based upon serologic testing; none of the brucellosis cases was based upon detection by aerobic blood culture. *C. neoformans* is *Clostridium neoformans; E. coli* is *Escherichia coli*; *C. burnetii* is *Coxiella burnetii*; *K. pneumoniae* is *Klebsiella pneumoniae; R. africae* is *Rickettsia africae*; *S. pneumoniae* is *Streptococcus pneumoniae*; *S. enterica* is *Salmonella enterica*; *S. pyogenes* is *Streptococcus pyogenes*; and *P. falciparum* is *Plasmodium falciparum.*

The distribution of CRP levels across the different aetiological groups is presented in Figure 7.2. Considering all bacterial infections, the median CRP level was 120 (46-224) mg/L, 173 (80-315) mg/L in bacterial BSIs, 108 (31-208) mg/L in bacterial zoonoses, 117 (78-195) mg/L in malaria, and 2 (1-14) mg/L in fungal BSIs. When considering patients without any pathogen identified, the median CRP level was 32 (5-109) mg/L among outpatients and 87 (28-191) mg/L among inpatients (p-value <0.001).

Two of 31 (6.5%) patients with a bacterial BSI, namely *Salmonella enterica* serovar Typhi, presented with abdominal pain and CRP concentrations below 10 mg/L: one had CRP measured at 1 mg/L and was discharged without receiving any antibiotic, while the other had a CRP measured at 12.5 mg/L and received Azithromycin before being discharged. Both were outpatients.

One inpatient (3.2%) classified in the bacterial BSI with a co-detection of *Klebsiella pneumoniae* and *Leptospira* spp. presented with an acute undifferentiated fever and CRP level below 40 mg/L, measured at 39.8 mg/L. This patient was treated by Chloramphenicol and discharged alive.

Considering bacterial zoonosis, 8 (13.1%) and 11 (18.0%) of 61 patients had CRP concentrations <10 mg/L and <20 mg/L, respectively. Brucellosis cases accounted for 8 (42.1%) of the 19 bacterial zoonoses with CRP levels below 20 mg/L, with a median of 8.8 (1.2-14.7) mg/L. Two of them declared antimalarial intake prior to attendance, two did not have any antibiotic prescribed after the medical consultation, none were prescribed either tetracycline, rifampicin, or streptomycin, and two received quinolone. Other antibiotics included metronidazole and amoxicillin-clavulanate. All of these brucellosis cases were discharged alive.

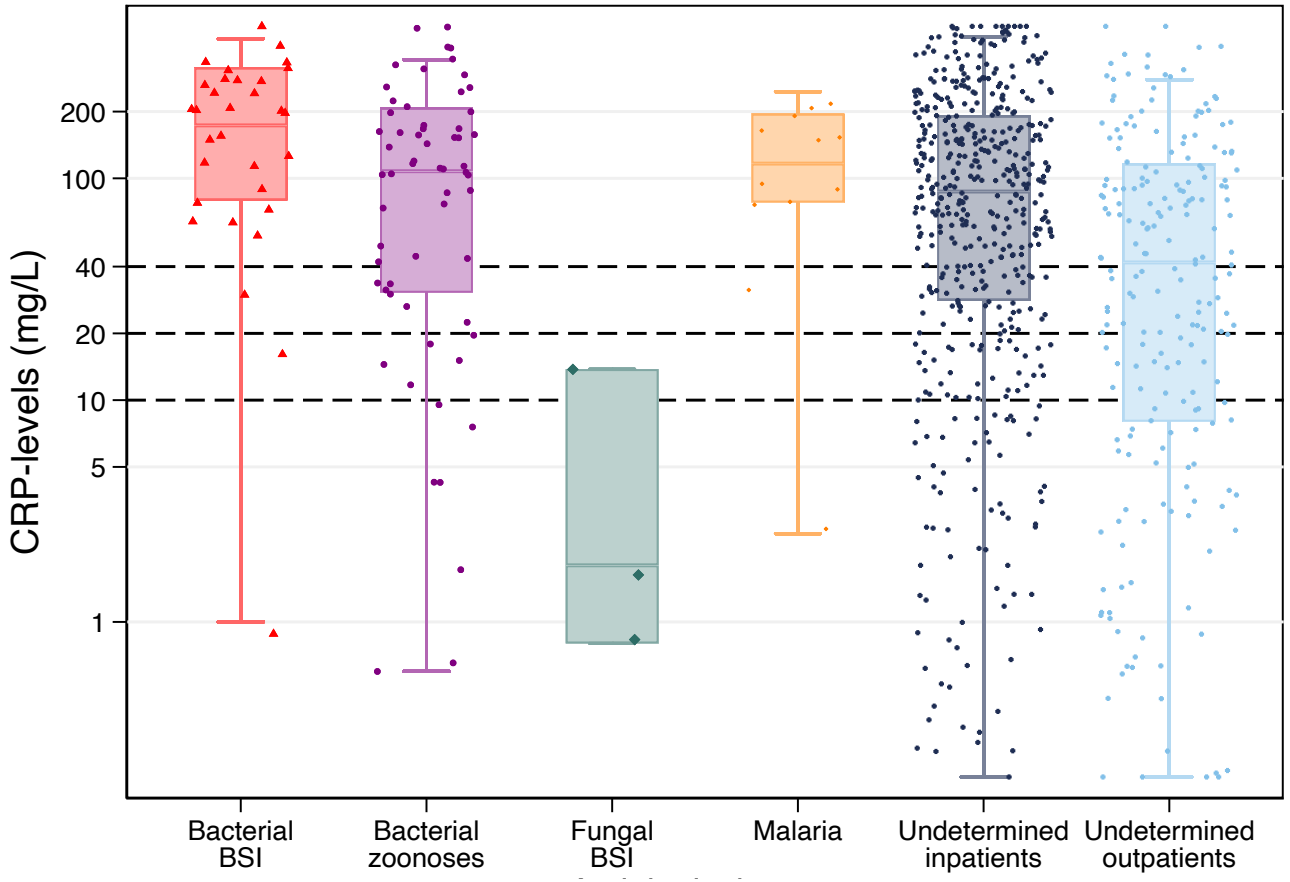


Figure 7-2 Distribution of CRP concentrations (log-scale) by aetiological group among febrile outpatients and inpatients, Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

CRP – C-reactive protein
BSI – Blood stream infection
Box boundaries shows 25th and 75th percentiles of the CRP-levels and line within the box shows the medians.
The Whiskers indicate the 10th and 90th percentile of the CRP-levels.

7.2.4 C-reactive protein performance

Findings of CRP performance in identifying bacterial infections are presented in Table 7.4. Considering all bacterial infections, CRP sensitivity ranged between 90% (84-96%) and 78% (70-87%) using a cut-off between 10 to 40 mg/L. Considering bacterial BSIs, sensitivity varied between 97% (83-100%) using a 10 mg/L cut-off and 90% (74-98%) using a 40 mg/L cut-off. Considering bacterial zoonoses, CRP sensitivity ranged between 87% (76-94%) using a 10 mg/L cut-off and 72% (59-83%) using a 40 mg/L cut-off.

Considering outpatients without any organism detected, 154 out 208 (74.0%) had an antibiotic prescribed. In case of prospective CRP-testing, prescription levels would have been reduced to 73.1% using a 10 mg/L cut-off, 63.5% using a 20 mg/L cut-off, and 51.0% using a 40 mg/L cut-off. Besides, antibiotic coverage would have been of 100% for CRP levels above each of these cut-offs.

Considering patients without organism detected and CRP levels above 40 mg/L, 21.7% (23/106) of outpatients were not covered by any antibiotic in routine care, while there were only 9.6% (33/344) among inpatients.

Considering the 235 outpatients with a broad-spectrum antibiotic prescription, CRP-guided treatment would have reduced it from 95% (40.4%) to 73% (31.1%), 63% (26.8%), and 50% (21.3%), using cut-offs of 10, 20 and 40 mg/L, respectively.

Table 7-4 Percent sensitivity (95% Confidence Intervals) of CRP at cut-off of 10, 20 and 40 mg/L by aetiological group among febrile outpatients and inpatients, Kilimanjaro Christian Medical Centre and Mawenzi Regional Referral Hospital, Tanzania, 2011-2014.

Aetiological Group	CRP >10 mg/L	CRP >20 mg/L	CRP >40 mg/L
All bacterial infections (n=92)	90 (84-96) 83/92	86 (79-93) 79/92	78 (70-87) 72/92
Bacterial BSI (n=31)	97 (83-100) 30/31	94 (79-99) 29/31	90 (74-98) 28/31
Bacterial zoonosis (n=61)	87 (76-94) 53/61	82 (70-91) 50/61	72 (59-83) 44/61
Fungal BSI (n=3)	33 (8-91) 1/3	0	0
Malaria (n=12)	92 (62-100) 11/12	92 (62-100) 11/12	83 (52-98) 10/12
Undetermined inpatients (n=490)	86 (83-89) 412/490	79 (75-82) 386/490	70 (66-74) 344/490
Undetermined outpatients (n=208)	73 (67-79) 152/208	64 (57-70) 132/208	51 (44-58) 106/208

CRP – C-reactive protein
BSI –Blood stream infection

7.3 Discussion

7.3.1 C-reactive protein performance

We demonstrated that CRP is elevated in more than 90% of patients with a confirmed bacterial BSI, and these data are a key step in examining the safety of CRP-guided therapy in sub-Saharan Africa. Based on these findings, further studies are warranted to examine the safety and clinical utility of CRP-guided therapy in low-level care, the setting where point-of-care CRP testing might have the largest impact in reducing unnecessary antibiotic prescription.

Two paediatric studies evaluated the diagnostic utility of CRP in detecting bacterial infections among febrile outpatients in Tanzania: Mahende et al. (2017) concluded that CRP could play a useful role in guiding antibiotic therapy if combined with a clinical evaluation, with an area under the receiver operator characteristic curve of 0.83 [433]. Only 17 patients had a blood-culture-confirmed though. Hildenwall et al. (2017) found CRP concentrations to be significantly higher in bacterial infections (median 40.1 mg/L) compared with non-bacterial illnesses, although only six children had a positive blood culture and two of them had CRP levels below 5 mg/L [209]. CRP levels in our outpatients were generally higher than both the latter two studies, as well as those in Keitel et al. (2017) where more than 91% of febrile children attending Tanzanian primary care clinics had CRP levels below 40mg/L [434]. This suggests to include different settings among the first lines of care, in order to capture the range of patient severity and pathogen exposure. Another study from Keitel et al. (2019) was a randomised non-inferiority trial with an endpoint of clinical failure at day 7, demonstrated that CRP POCT and additional diagnostic interventions integrated within an electronic care algorithm safely reduced antibiotic prescription among children attending the first levels of care with an acute respiratory infection [211]. Collectively, all these studies consistently indicate that CRP has the potential to safely reduce unnecessary antibiotic prescription in the paediatric primary care setting. As febrile adults constituted 92% of patients in our cohort, our study complements these findings among paediatric populations by demonstrating that CRP may also have a useful role in guiding antibiotic prescribing in older patients.

In our cohort, more than half of outpatients with an antibiotic were prescribed a broad-spectrum one. The likelihood of a broad-spectrum antibiotic prescription among outpatients did not vary based upon CRP concentrations, suggesting a widespread tendency among health workers to favour broad-spectrum agents. Broad-spectrum antibiotics are a major contributor to antimicrobial resistance [354], and CRP has recently been demonstrated to reduce such prescription among patients attending primary care in Southeast Asia [100]. Among our outpatient study population, implementing a conservative prescribing strategy using a 10 mg/L cut-off would have reduced broad-spectrum antibiotics from 40% down to 31%; and a 40 mg/L cut-off would have reduced their prescriptions by approximately half, from 40% down to 21%. Conversely, antibiotic coverage was missing in more than 20% of outpatients whereas CRP levels were above 40 mg/L. This suggests that CRP may not only reduce unnecessary antibiotic prescription, but also help identify patients who really need an antibiotic.

In the bacterial zoonotic group, we found that CRP had moderate sensitivity. This finding should be interpreted in regard with the small number of cases for each zoonotic organism under study. Of note, CRP was elevated among the bacterial zoonotic cases (median 108 [IQR 31-108] mg/L), and the CRP thresholds captured most cases of leptospirosis, Q fever and spotted fever rickettsiosis cases. Eight of the eleven bacterial zoonoses with CRP levels below 20 mg/L were brucellosis cases. Given that *Brucella* is an intra-cellular pathogen with mechanisms of immune-evasion, brucellosis might elicit only a mild to moderate inflammatory response [435]. These corresponding findings among brucellosis cases should be verified in other cohorts before making conclusions on CRP performance for this zoonotic disease. Similarly, while our findings for the other three bacterial zoonoses look promising, further study is warranted for each of those pathogens.

7.3.2 Limitations

A limitation of our study is the absence of viral investigations, preventing the determination of CRP specificity. In studies investigating causes of fever and evaluating corresponding CRP performance in Southeast Asia, CRP was found to have a specificity ranging 67-72% using a 20 mg/L cut-off [316, 427]. However, it is noteworthy that CRP levels in the current study were relatively high compared with similar studies done in Southeast Asia [107], and this may have lowered specificity even

more. Among the children enrolled, most had insufficient blood volume available for CRP-testing; accordingly, adults comprised over 92% of patients in the current analysis, and conclusions drawn from this analysis are most relevant to febrile adults.

HIV infection status was not confirmed in our study population, so we were unable to assess CRP performance among patients living with HIV. A prior study in Malawi demonstrated that CRP does rise in persons living with HIV found to bacterial, mycobacterial or fungal infections [128]. While this suggests consistent performance regardless of the HIV status, further studies are warranted in this particular population.

Chapter 8

GENERAL DISCUSSION & CONCLUSION

8 GENERAL DISCUSSION & CONCLUSION

8.1 C-reactive protein for distinguishing bacterial from viral pathogens

This Thesis aimed to identify non-malarial pathogens causing acute fever among non-severe patients attending primary care in Southeast Asia, and to evaluate whether C-reactive protein (CRP) could distinguish bacterial from viral pathogens.

In Southeast Asia, the CRP Study mainly detected dengue virus, influenza virus type A, respiratory syncytial virus (RSV) and *Leptospira* spp. as key contributors, in line with previous studies in the region [21, 107]. Regarding the inclusiveness of our study criteria, our findings may be applicable to the general population, regardless of age, comorbidity or clinical presentation.

The evaluation of CRP for the distinction between bacterial and viral pathogens among our non-severe patients in primary care showed limited performance, precluding any major clinical utility. The *princeps* study in the region showed a greater accuracy [107], but this difference requires further comment: more than half of these patients were hospitalised; the detection of bacteraemic patients by blood culture indicates the presence of serious infections requiring an antibiotic. In our study, pathogens were mainly detected by molecular assays, for which pathogenicity may not be fully assumed. Moreover, our CRP evaluation dealt with a small number of pathogens with low inflammatory levels, and all patients recovered regardless of the aetiology or antibiotic prescription. It is thus possible that most infections -bacterial or viral- were self-limiting in the CRP Study, while serious bacterial infections remained an exception. This scenario has recently been confirmed in Tanzania, where only 2.3% of febrile children attending primary care received an antibiotic without altering their clinical outcome [211]. Strategies using CRP for discriminating self-limiting from serious infections may therefore be more relevant than those aiming to distinguish between bacterial and viral pathogens.

In our study, the absence of a control group undermined the evaluation of CRP for distinguishing between bacteria and viruses: some organisms we detected may have also been found among healthy controls, especially those in nasopharyngeal (NP) swabs, as distinguishing between

commensal microbiota and pathogenic organisms is challenging, particularly among children [436]. This explains why we only attributed pathogenicity to organisms consistently detected among cases rather than healthy controls in the literature [368, 372, 373]. But despite adopting large sample sizes with prolonged follow-up and gold standard diagnostic methods, all these case-control studies admitted limitations about ascertaining causality for all cases, as a certain degree of colonisation has been described for all organisms [374].

In Moshi, northern Tanzania, our CRP evaluation focused on febrile patients with a culture-confirmed bacterial infection or with serological evidence. Non-malarial pathogens are poorly studied in sub-Saharan Africa compared to Southeast Asia, despite the high burden of infectious diseases in the region [2, 23, 121, 437]. Health workers face more and more the challenge of identifying febrile patients with a negative malaria rapid diagnostic test (RDT) who need an antibiotic, and scenarios of increased prescribing levels in case of introduction of malaria RDTs have been reported [438]. Antibiotic use is thus irrational with prescribing levels exceeding 80% in some primary health care settings [99, 255], while access to antibiotics is limited in some regions causing a greater mortality than antibiotic resistance [439]. In the literature, CRP assessments are limited and target a single pathogen [123, 124, 187-190, 212, 215, 216, 223, 226, 233]. The few studies investigating the whole range of pathogens causing acute fever with corresponding CRP levels are centralised in urban hospitals, implying the same limitations found in Southeast Asia that prevented us from generalising findings to patients attending low-level care [129, 130, 209, 224]. One of the strengths of our study was the inclusion of both hospitalised and ambulatory patients, informing about pathogen spectrum and corresponding CRP performance in different types of healthcare settings and patient severity. This also implies that our findings were different from the CRP Study, where exclusively primary care and non-severe patients were recruited.

The combination of blood culture and bacterial serological assays allowed the detection of a whole range of organisms including zoonotic pathogens, and of these, *Leptospira* spp. and *Brucella* were the most common. *Escherichia coli* and *Salmonella enterica* serovar Typhi were the most prevalent pathogens found in blood culture, whereas *Plasmodium falciparum* was only detected in a

minority of cases, confirming the decline of malaria as the leading cause of acute fever in sub-Saharan Africa [23, 120, 121].

CRP sensitivity was high for detecting bacteria in blood stream infections (BSIs), and moderate considering bacterial zoonoses. This last finding should be interpreted with caution: the number of each bacterial zoonotic pathogen was low which limits the generalisation to zoonotic illness in general. Most *Brucella* cases presented with low CRP levels, while other zoonotic pathogens like *Leptospira* or *Coxiella* showed higher inflammatory levels. This warrants further evaluation with sufficient cases of each zoonotic pathogen.

A major limitation in the full evaluation of the CRP performance in this study was the absence of viral investigations: specificity was thus not measured, as some negative cases may be undetected bacterial pathogens. Given that specificity usually does not exceed 90%, it is therefore possible that some patients with elevated CRP concentrations may actually correspond to viral infections [316, 427]. The benefit of the CRP use to ensure maximum antibiotic coverage of bacterial infections -particularly blood stream infections- with the disadvantage of unnecessarily treating a few viral infections should be considered according to the local context.

Finally, aiming to distinguish bacterial from viral pathogens implies similar limitations as those described in the CRP Study in Southeast Asia: some of the organisms detected -especially among non-severe ambulatory patients- may not be pathogenic. We identified two Tanzanian outpatients with confirmed *Salmonella enterica* serovar Typhi who presented with CRP levels below 10 mg/L with only one receiving an antibiotic and both being discharged. Another Tanzanian study found two out of six outpatients having CRP levels below 5 mg/L despite a positive blood culture [209]. Demonstrating whether CRP missed pathogenic bacteria or ruled-out a self-limiting infection is challenging, and this question may remain unanswered regardless the sensitivity of microbiological investigations.

8.2 C-reactive protein for guiding antibiotic prescription

A key research focus of this thesis aimed to evaluate whether CRP-testing at point-of-care (CRP POCT) could prospectively guide antibiotic prescription without altering patient clinical outcome, regardless of the aetiology. More specifically, this strategy intended to understand whether CRP POCT could rule-out antibiotic prescription for patients presenting with a self-limiting infection, rather than differentiating between bacterial and viral pathogens. Evidence here again originates from high-income countries mainly, targeting patients with a respiratory tract infection [79, 260-265]. In low-to middle-income countries (LMICs), only a few studies prospectively demonstrated CRP POCT could significantly reduce antibiotic prescription, and only among patients with a particular age category and/or respiratory symptoms and/or with additional point-of-care diagnostic tools [99, 274]. This Thesis extended the evaluation of CRP POCT to all febrile patients attending primary care regardless of their age or clinical presentation.

In the CRP Study, we prospectively tested CRP POCT and demonstrated a moderate but significant antibiotic reduction using a 40 mg/L threshold without altering patient clinical outcome. This reduction remained significant when analysing adults separately, as well as in the subgroup of patients with either a neurological or respiratory presentation or among those with a documented fever. In the literature, febrile patients attending low-level care often present with respiratory symptoms, for which CRP POCT has shown significant reduction [211, 274], and our study was in line with these findings. We also reported a significant reduction considering the prescription of broad-spectrum antibiotics: although these should be restricted to hospitals targeting the most severe patients, we still observed such prescriptions among our non-severe ambulatory patients. Several publications have demonstrated broad-spectrum antibiotics -particularly in monotherapy- to represent a key driver of antibiotic resistance, and this reduction may represent an additional benefit for mitigating this burden at the community level [440].

A major comment to these findings relates to lower routine prescribing levels among the control group at the time of the CRP Study compared to the baseline survey. Our study used an individual

randomisation design, and it is likely that health workers progressively realised that most patients from the intervention groups were classified as "low" CRP levels with the recommendation to withhold antibiotics. The same health workers may have lowered their prescriptions for similarly non-severe patients from the control group, although no CRP result was informed. This contamination, combined with the Hawthorne effect due to the presence of the research team onsite, probably affected routine prescribing behaviours. Future evaluations with the exclusive presence of the routine staff using the test should be considered, as well as the adoption of a cluster randomised trial design using the health centre as a unit of randomisation.

Another limitation of this study related to safety: although clinical outcomes were similar between intervention and control groups, the CRP Study was not powered to detect a difference in recovery between these groups. Regarding the low risk of any severe outcome in low-level care where only non-severe patients were recruited, we would not have been able to reach a sufficient sample size to demonstrate the safety of antibiotic reduction.

Finally, we did not adopt a prescriptive guidance towards CRP results: health workers were recommended to keep using their clinical judgment for deciding whether an antibiotic was necessary, and CRP was only provided as additional information in the intervention groups. In line with the literature, we do not support the use of a biological test on its own without any clinical assessment. In our study, all febrile patients were tested for CRP and benefited from a clinical examination for deciding about antibiotic prescription. Recent publications suggested to restrict CRP-testing to patients at risk to develop serious infections, implying a preliminary clinical screening [211, 286]. In settings where routine antibiotic prescription levels are low with trained medical physicians and functioning follow-up and referral systems, the objective may be to improve the identification of the few primary care patients at risk of a serious bacterial infection. In this scenario, the use of a high threshold (e.g. ≥80 mg/L) may reinforce this strategy by limiting the number of false positives. As an illustration, a primary care-based study in Norway did not find any benefit in testing all febrile children with CRP, either in antibiotic reduction nor in referral to hospital [286]. On the other hand, in settings where routine prescribing levels are high with minimally trained health workers and hard-to-reach patients, a strategy testing all febrile patients using a low CRP threshold for ruling-out self-limiting infections may already

reduce prescription levels, while ensuring a certain layer of safety [34, 234, 291]. The local context should therefore be primarily considered when designing a strategy using CRP, especially in terms of access to care and routine prescribing behaviours.

In sub-Saharan Africa, we were not able to prospectively evaluate the benefits of CRP POCT, but considering the routine prescribing habits, testing all febrile patients would have significantly reduced antibiotic prescription, even when using a low threshold of 10 mg/L. It is noticeable that around half of our outpatients -not severe by definition- with an antibiotic prescription received a broad-spectrum antibiotic, and this particular prescription did not vary across CRP concentrations. At this primary level of care, such prescribing behaviour illustrates the need to help primary health workers in better targeting febrile patients who need an antibiotic. Furthermore, all the thresholds evaluated from 10-40 mg/L showed sensitivities equal to or above 90% for detecting bacterial BSIs, suggesting preliminary evidence for safety in reducing antibiotic prescription.

8.3 Strategies for improving fever management

Identifying key pathogens causing acute fever remains critical to inform empiric treatment guidelines: although patient management is mainly based on clinical examination in primary care, evidence on the local epidemiology can help health workers in refining their antibiotic strategy.

Prior to this step, identifying patients who need an antibiotic represents a fundamental challenge. Host-response biomarkers tested at point-of-care can directly support health care workers in this decision at the time of the consultation. Non-severe patients often present with self-limiting infections that resolve without antibiotic regardless of the aetiology, questioning strategies aiming to distinguish between bacterial and viral pathogens. Besides, some bacteria and viruses included in biomarker evaluations are systematically considered causative, whereas pathogenicity cannot be ascertained for all cases, especially in NP swabs [368]. Finally, microbiological investigations used for biomarker assessment rarely originate from low-level care and even less from LMICs, where extensive laboratory capacities and skilled microbiologists are limited. This is illustrated by the fact that most biomarkers are assessed on pathogens detected in tertiary hospitals in high-income countries [361]. Biomarker performance may therefore not apply to non-severe patients in the tropics.

Several studies yet pursued this strategy of distinguishing between bacterial and viral pathogens, and chose to associate CRP with similar host-response biomarkers for improving diagnostic accuracy. For instance, a few evaluations reported high performance when CRP was combined with biomarkers of viral infections, such as TNF-related apoptosis-inducing ligand (TRAIL), Myxovirus resistance protein (MxA) or Interferon-γ-inducible protein 10 (IP-10) [70, 441-444]. The same limitations apply here regarding the difficulty to ascertain the pathogenicity of the organism detected, bearing in mind that evaluations of these innovative tests were not carried out in the tropics nor in low-level care. Nonetheless, these tests are currently not available at point-of-care and still require laboratory resources, undermining their potential implementation among minimally trained health workers in resource-poor settings. Incremental diagnostic accuracy of these combined tests should be carefully weighed against their additional cost [445].

Considering all these limitations particularly prevalent in the context of LMICs, the strategy of evaluating host-biomarkers as a clinical tool discriminating between self-limiting and serious infections based on clinical recovery regardless of the aetiology may be of relevance. Following this approach, a few studies have demonstrated CRP POCT to significantly reduce antibiotic prescription among febrile but non-severe patients attending primary care in LMICs [99, 100, 211, 274]. A common shortcoming of CRP is an imperfect specificity for thresholds usually selected (i.e. between 10-40 mg/L), implying that some patients with a self-limiting or a severe viral infection may be indicated as requiring an antibiotic [107, 446]. Selecting a high threshold (e.g. 80 mg/L) for limiting false positives would, however, imply safety concerns: a systematic review reported a specificity over 90% when using such high threshold, with however, a corresponding sensitivity inferior to 50%, likely to miss some potentially lethal bacterial infections [446]. This trade-off between sensitivity and specificity should be weighted according to the local context. In primary care settings where prescribing levels are well-regulated with highly-trained and experienced staff with accessible referral system, the biomarker impact may be maximised when patients are preliminary screened clinically for selecting those at risk, and using a high threshold would help ruling-in the very few with a serious bacterial infection. This strategy has been validated in Europe [358, 447]. However, settings with high prescription levels and inconsistent referral systems where patients live in hard-to-reach areas may opt for an extended testing to all febrile patients, using a low threshold (i.e. below 40 mg/L). This could be summarised by two distinct questions:

- Is the objective to identify the very few patients with a serious infection?
- Or is it to reduce antibiotic prescription by ruling-out the majority of self-limiting infections?

This strategy aiming to help primary health care workers in predicting whether an infection is self-limiting or serious suggests CRP to predict severity. Evidence is, however, contrasted: a hospital-based study in the UK reported a high CRP performance for predicting meningococcal disease among children presenting with fever and rash, with an area under the receiver operator characteristic curve (AUC) at 0.90 (95% CI 0.83-0.97) [448]. In the context of LMICs, a single study evaluated several endothelial and immune activation biomarkers for predicting 28-day mortality among 507 acutely

febrile adults in Tanzanian primary care [449]. Prognostic accuracy was enhanced when clinical scoring systems were combined with one of the biomarkers, namely the soluble triggering receptor expressed on myeloid cells (sTREM-1), while CRP was of a limited utility (area under the receiver operating characteristics AUROC for bacterial infection 0.89, 95% CI [0.73-0.95] *versus* 0.51 [0.34-0.69], p-value <0.001, respectively). CRP concentration was significantly lower among non-survivors than the survivors (median 24.8 [7.8-80.0] *versus* 52.5 [18.7-143.3], p-value 0.021, respectively). Further, 75% of the Tanzanian survivors had CRP levels below 80 mg/L, which is consistent with the literature where most serious infections are detected above this concentration [446]. On the other hand, 25% of the Tanzanian non-survivors had CRP-levels below 20mg/L, indicating CRP to be an imperfect marker for prognosing mortality among febrile patients.

These two latter studies evaluated CRP for predicting severe outcomes such as organ failure or death, which was not the objective of this work. The CRP Study stands in the context of non-severe outpatients and was originally meant to identify febrile patients who need an antibiotic. Because most patients recovered regardless whether an antibiotic was prescribed or not, and regardless of the organism detected, this Thesis indicates that low CRP levels may correspond to self-limiting infections, while greater concentrations may not ascertain the presence of a serious infection [100, 446]. This evidence represents an attractive prospect for future primary care-based studies, which is to evaluate whether CRP could predict -in combination with a basic clinical examination- the need for higher levels of care, and not only the need for an antibiotic.

More research is therefore warranted to pursue the evaluation of CRP for predicting severity in various levels of care and not restricted to mortality. In 2020-2021, one study will extend the Tanzanian findings on biomarkers -including CRP- predicting severity to all febrile children, based in Southeast Asia [450]. Parallelly, another study based in rural Vietnamese will evaluate the impact of CRP POCT on antibiotic prescription with a limited research environment among primary care patients with an acute respiratory tract infection [451]. This intervention will adopt a cluster-randomised design and consist in delivering a 5-minute training for a simple lateral flow device, based on a semi-quantitative method with thresholds <10 mg/L; 10-40 mg/L; 40-80 mg/L and >80 mg/L.

Apart from the specific biomarker to adopt, additional interventions should be integrated: Thailand, for instance, successfully reduced prescription levels in primary care through the implementation of key indicators on prescription objectives with financial incentives and health inspectors [289]. Although it is challenging to accurately quantify the actual impact of such policy, reports from Southeast Asia consistently rank Thailand with lower prescription levels compared to Myanmar for instance, where policy tends to favour antibiotic access rather than regulation [34]. Additional interventions have been validated in primary care, including evidence-based antibiotic stewardship programmes; health workers information and communication skills training [452, 453]. These should also be implemented, in order to maximise changes in prescribing behaviours.

8.4 Conclusion

This Thesis examined the use of CRP-testing to guide primary health workers in Southeast Asia in their decision to prescribe antibiotics to febrile patients. This approach has been widely used in many high-income settings, particularly in hospitalised patients. Over the past ten years the evidence to support the use of CRP POCT has extended to LMICs, where the burden of febrile illness is considerable. More evidence, however, was needed from pragmatic evaluations to confirm the impact of CRP POCT in routine practice.

We considered the utility of the tests in two areas: first, in the CRP Study we primarily focused on CRP testing as a clinical tool with the objective to improve antibiotic prescription based on patient clinical recovery, by ruling-out self-limiting infections. To this end we found a modest but significant impact on antibiotic prescription, with lower overall prescribing, and more rational targeting with respect to CRP concentrations. This represents one of the key findings. The second measure of utility was microbiological, where we examined CRP as a discriminator between bacterial and viral pathogens in febrile patients in primary care. While we found CRP levels to be higher in bacterial infections as compared with viral ones, the overall performance was lower than in other studies in the region. In a second cohort from Tanzania we found CRP levels to be consistently high in patients with bacterial infections, but in the absence of viral diagnoses the assessment of the performance of CRP was limited.

The management of non-malarial febrile patients in primary care is challenging, and no diagnostic tool perfectly identifies those who really need an antibiotic. Consequences of irrational antibiotic use have already started to aggravate febrile illness morbidity and mortality with the emergence of bacterial resistant strains, and prescribing practices in many LMICs are an acknowledged driver of this silent but lethal outbreak [305]. Not only overuse but also paucity of antibiotics exacerbates the burden of febrile illness, and interventions should not favour restriction at the expense of access. Antibiotics are a precious resource that saved millions of lives since their discovery in 1928, and restricting access without providing additional diagnostic tools guiding fever management may understandably be perceived as risky from both health worker and patient perspectives.

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APPENDICES

APPENDIX I

Standard operating procedure (SOP) for C-reactive-testing at point-of-care

Aim

This SOP provides the instructions for determining the C-reactive protein (CRP) level from human whole blood, plasma, or serum using the NycoCard Reader II CRP point-of-care test in the laboratory or in the field.

Scope / Responsibility

Study staff working at a satellite research unit or at MORU, under the supervision of the unit/study lead and under the auspices of the MORU biological safety officer (Dr Stuart Blacksell).

The unit/study lead is responsible for supervising the use of this SOP by laboratory and clinical staff, in accordance with biosafety requirements.

This protocol involves the use of human samples. All users MUST have health and safety induction training as per COSHH prior to performing laboratory procedures as outlined in this SOP.

Coshh summary

Location of work: Microbiology/CCRU	**Persons involved:** Other, specify: Areerat Thaiprakhong, Nattapon Pinthong, Tri Wangrangsimakul, Jeroen Bok
PPE required ☒ Gown ☒ Apron ☐ Respiratory mask ☐ Blue gown (BSL3) ☒ Gloves ☒ Sleeves ☐ Full resp. mask ☐ Doubble glove (BSL3) ☒ Goggles ☐ Face shield ☐ Bio spill clean up ☐ Hair net (BSL3) ☒ Cover shoes ☐ Cryo Gloves ☐ Chem spill clean up ☒ work in BSC	
Hazards identified	
All hazards identified in the COSHH evaluation are incorporated/covered in the general safety training provided regularly to staff (see Safety Manual) and controlled by the use of PPE selected (see COSHH evaluation). No particular hazards that require additional warning or point of care are identified in this Procedure.	
Waste disposal procedures:	
Clean up following standard procedure for working with organism in biosafety cabinet (see Safety Manual). Waste disposal is collected as and handled separately according to local site waste disposal SOP.	

Methods

Pre-processing

General points:

General laboratory and clinical biosafety and security procedures should be maintained at all times including the appropriate use of personal protective equipment (PPE)

In the laboratory, always process samples in the class II biological safety cabinet under sterile conditions. In the field, use the aseptic non-touch technique to obtain blood and process samples

Stability and storage of the NycoCard Reader II CRP POCT kits:

Unopened kits must be stored in the 4^{o}C refrigerator and avoid exposure to direct sunlight and temperatures >25^{o}C. Do not freeze

Opened kits must be stored in the 4^{o}C refrigerator when not in use to maintain stability of reagents. Do not freeze and avoid exposure to direct sunlight and temperatures >25^{o}C

Quality control:

The CRP measuring range using the NycoCard Reader II is 8-200mg/l for whole blood and 5-120mg/l for serum or plasma

The Afinion CRP control solution should be used to confirm the efficacy of the reagents and correct performance of the test

Substitute the control solution for a patient sample according to this SOP

For reading the result with the NycoCard Reader II CRP POCT, select the "CRP Serum/Plasma" option on the menu and read twice to ensure within-test precision and record results on the QC log

The measured value should be within the acceptance limits on the vial label

The quality control procedure should be performed twice (to ensure between-test precision) on opening of each new reagent kit with results recorded in the QC log along with lot/batch number of test kit, location, date and identity of the person performing the QC. An acceptable range of variation between control tests is +/- 6.5%

If the measured QC value falls outside the acceptance limits:

- repeat the procedure with a different user,

- then repeat using a new kit if QC failure is persisting,
- then repeat using a different NycoCard Reader II CRP POCT if QC failure is persisting

If QC failure occurs in the field, the NycoCard Reader II CRP POCT machine should be returned to the laboratory for repeat QC testing in a more controlled environment

Materials required:

- Disposable gloves, laboratory coat, laboratory goggles/glasses
- Disposable sterile pipette tips
- Pipette set
- Disposable transfer pipettes or 50µl MiniPet and pipette tips
- Permanent marker for sample labelling
- NycoCard Reader II CRP POCT kits containing 5µl capillary tubes, capillary tube holders or forceps, R1/Dilution liquid, R2/Conjugate, R3/Washing solution, Control solution, TD/Test device

Processing

Equipment required:

- Class II biological safety cabinet (laboratory only)
- Vortex
- NycoCard Reader II CRP machine
- 4˚C refrigerator

In the BSL-2 laboratory:

1. Bring the test tube(s) with the R1/Dilution liquid to room temperature before use. R2/Conjugate, R3/Washing solution and TD/Test device can be used cold or at room temperature
2. Label the R1/Dilution liquid tube(s) and TD/Test device with the study subject ID
3. Pipette 5µl of EDTA venous whole blood (or plasma/serum sample or control solution) and add it to the R1/Dilution liquid tube.

4. Close the R1/Dilution liquid tube lid and vortex to mix for 10 seconds
5. Pipette 50µl of diluted blood sample (or plasma/serum sample or control solution) and apply it to the TD/Test device without touching the membrane with the pipette tip
6. Allow the sample to soak into the membrane for 30 seconds
7. Apply 1 drop of the R2/Conjugate to the TD/Test device, holding the droplet bottle vertically and 1cm above the membrane
8. Allow the conjugate solution to soak into the membrane for 30 seconds
9. Apply 1 drop of the R3/Washing solution to the TD/Test device, holding the droplet bottle vertically and 1cm above the membrane
10. Allow the washing solution to soak into the membrane for 30 seconds
11. Read the result within 5 minutes using the NycoCard Reader II CRP machine
12. Switch the machine on
13. Go to "Main menu/Enter test" and press ENTER
14. Whole blood samples: Go to "CRP Whole blood" and press ENTER
15. Serum/plasma samples or control solution: Go to "CRP Serum/Plasma" and press ENTER
16. When "…Place pen" is displayed, place the pen on the test device and push down the pen sleeve
17. Hold the pen still until the result is displayed
18. Record the CRP result in the sample record and shipment log (or, if performing quality control, in the quality control log)
19. Return the NycoCard Reader II CRP POCT kit to the 4ºC refrigerator

<u>In the field</u>:

1. Bring the test tube(s) with the R1/Dilution liquid to room temperature before use. R2/Conjugate, R3/Washing solution and TD/Test device can be used cold or at room temperature
2. Label the R1/Dilution liquid tube(s) and TD/Test device with the study subject ID
3. Obtain a finger-prick blood sample
4. Pick the middle or ring finger

5. Clean the area at the side of the ball of the finger, perpendicular to the lines of the fingerprint, with cotton wool and 70% ethanol
6. Use a lancet to prick the cleaned area and squeeze the finger to express 1 drop of capillary blood
7. Dispose of the lancet in a sharps box
8. Fill a 5µl capillary tube with the capillary blood using a capillary tube holder or forceps, avoiding air bubbles in the capillary tube and excess blood sample on the outside. If performing quality control, fill the capillary tube with the control solution instead
9. Drop the filled capillary tube into the R1/Dilution liquid tube and close the lid
10. Gently shake/mix well for 10 seconds or until the solution is clear
11. Pipette 50µl of diluted blood sample (or control solution) using the transfer pipette or MiniPet and apply it to the TD/Test device without touching the membrane with the pipette tip
12. Allow the sample to soak into the membrane for 30 seconds
13. Apply 1 drop of the R2/Conjugate to the TD/Test device, holding the droplet bottle vertically and 1cm above the membrane
14. Allow the conjugate solution to soak into the membrane for 30 seconds
15. Apply 1 drop of the R3/Washing solution to the TD/Test device, holding the droplet bottle vertically and 1cm above the membrane
16. Allow the washing solution to soak into the membrane for 30 seconds
17. Read the result within 5 minutes using the NycoCard Reader II CRP machine
18. Switch the machine on
19. Go to "Main menu/Enter test" and press ENTER
20. Finger-prick blood samples: Go to "CRP Whole blood" and press ENTER
21. Control solution: Go to "CRP Serum/Plasma" and press ENTER
22. When "…Place pen" is displayed, place the pen on the test device and push down the pen sleeve
23. Hold the pen still until the result is displayed
24. Record the CRP result in the CRF (or, if performing quality control, in the quality control log)
25. Return the NycoCard Reader II CRP POCT kit to the 4^{o}C refrigerator

Post-processing

Disinfection and waste disposal:

In the laboratory:

Dispose of the excess sample and used materials as per site

Tidy the work area in the BSCII and disinfect with 1% Virkon followed by 70% ethanol

In the field:

Place all used materials/biological waste in a designated waste bag and tie

Disinfect work surface with 70% ethanol

Transport waste (biological waste bag and sharps box) back to the main laboratory site for further disposal as per site.

Schematic overview of the NycoCard Reader II CRP point-of-care test

Laboratory - obtain 5µl EDTA whole blood from blood collection tube
Field – obtain 5µl whole blood from finger-prick using a capillary tube from the kit

1

Dilute sample:
Laboratory – pipette 5µl EDTA whole blood into R1/Dilution liquid tube
Field – drop the filled 5µl capillary tube into the R1/Dilution liquid tube

Close the lid, gently vortex/mix for 10 sec or until the solution is clear

2

Apply diluted sample:
Apply 50µl diluted sample to the TD/Test device
Allow to soak into the membrane for 30 seconds

3

Apply R2/Conjugate:
Apply 1 drop R2/Conjugate to the TD/Test device
Allow to soak into the membrane for 30 seconds

4

Apply R3/Washing solution:
Apply 1 drop R3/Washing solution to the TD/Test device
Allow to soak into the membrane for 30 seconds

5

Read the result (within 5 min):
Go to "Main menu/Enter test" and press ENTER
Go to "CRP Whole Blood" and press ENTER
When "…Place pen" is displayed, place the pen on the test device and push down the pen sleeve
Hold the pen steady until the result is displayed
Record the result and replace the pen in the holder

APPENDIX II

Standard Operating Procedure (SOP): Sample Collection – Dried Blood Spot (DBS)

Purpose and Scope

To collect whole blood samples from febrile patients consulting primary health care settings and Outpatient department (OPD), and enable nucleic acid detection of alpha & flavivirus. To minimize distress, a small volume of blood is taken by pricking the patient's finger and spotting this onto a filter paper.

Duties and Responsibilities

- Collect blood samples as described here in this SOP, using universal safety precautions
- Appropriately label and store samples until transportation
- Maintain the sample transfer log Materials and Equipment

The following materials are provided together with this SOP to collect samples:

1. Alcohol swab (Figure 1A, can be replaced by cotton wool with alcohol)
2. Cotton pad/wool (Figure 1B)
3. Sterile, disposable lancets (Figure 1C)
4. Sealable plastic bag (Figure 1E)
5. Whatman 31 ET Chr paper, cut in 7.5 x 3 cm. (Figure 1F)
6. Subject's Id sticker (Figure 1G)
7. Desiccant packs (Figure 1H)
8. Sample manifest

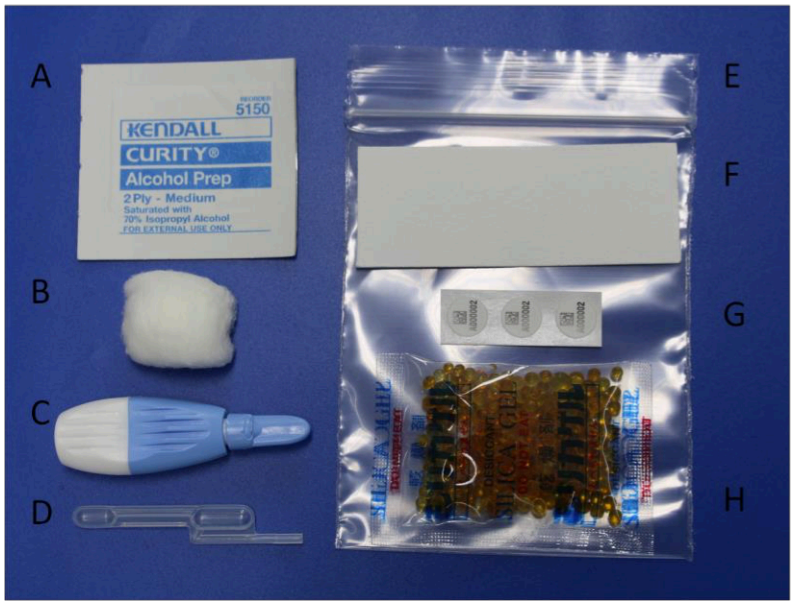


Figure 1 Materials and equipment used to collect DBS

Procedure for collecting samples

Step 1 At the time of sample collection, clearly explain to the patient and/or their guardian (this may be an accompanying relative or friend) what sample is required and how it will be collected.

Step 2 See Figure 2. Wear a new pair of disposable gloves. Use an alcohol swab to clean the finger before puncturing it (Figure 2A). Allow the skin to dry.

Step 3 Twist open the sterile lancet (Figure 2B). Press the lancet toward the finger to puncture the finger, a little off the centre of the finger pad (Figure 2C). Discard the lancet after use.

Step 4 Wipe away the first drop of blood with dry, clean cotton wool and apply gentle pressure to the finger (Figure 2D). Discard cotton wool after use.

Step 5 Drop one drop of blood onto the filter paper (Figure 2E-F).

Step 6 Repeat twice to blot the second and third blood spots. Blood should be applied only from one side of the filter paper. Make sure the blood soaks through to the other side of the filter paper, the blood spot should be roughly the same size on both sides of the paper.

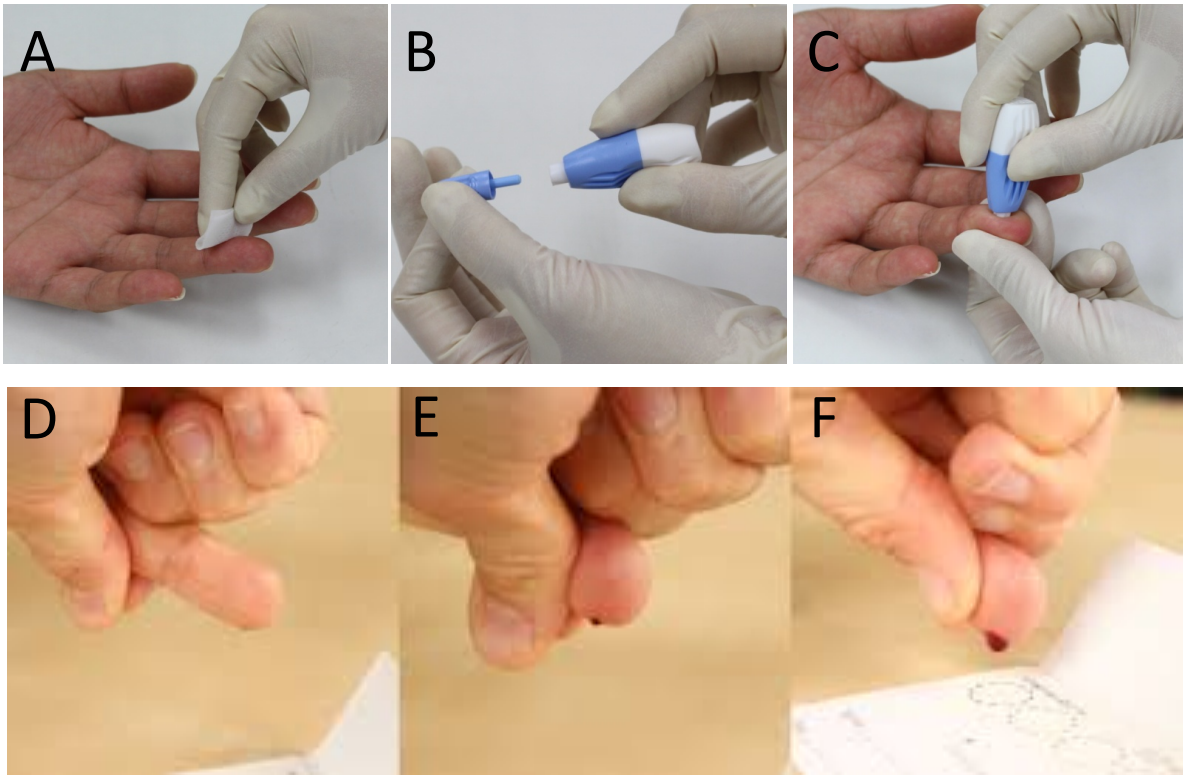


Figure 2 Finger prick and DBS collection procedure

Step7 Write the location and date of sample collection on the sample manifest.

Step 8 Place the stickers onto the filter paper, and place another one from the same set on the sample manifest.

Step 9 Allow the blood to air-dry overnight at room temperature. Do not let blood spots touch other samples during the drying process.

Step 10 Once completely dry, place each filter paper into a separate plastic bag, together with a sachet of desiccant (Figure 3). Seal the bags and store in a cool, dry place. Keep away from direct sunlight.

ONE PATIENT = ONE FILTER PAPER DO NOT SPOT SAMPLES FROM TWO PATIENTS ONTO THE SAME FILTER PAPER

Figure 3 Packing the DBS filter paper

APPENDIX III

Literature review – Search strategy

Query: What are the causes of fever in South-East Asia & Sub-Saharan Africa?

Search String

(((((("Fever"[Mesh]) OR "Sepsis"[Mesh])) AND (((((("Bacterial Infections"[Mesh]) OR "Virus Diseases"[Mesh]) OR "Bacteremia"[Mesh]) OR "Zoonoses"[Mesh]) OR "etiology"[Subheading])) AND "Asia, Southeastern"[Mesh])

Filters used

- Published – since 1st January 2015 - 31st April 2019
- Article type: Clinical Study; Clinical Trial; Comparative Study; Controlled Clinical Trial; Journal Article; Multicentre Study; Observational Study; Pragmatic Clinical Trial; Randomized Controlled Trial
- English Language
- Species: humans

Excluded

- Studies dealing with travellers
- Respiratory presentations only without fever
- No microbiological investigations, or not informed
- Mathematical modelling studies
- Case report
- Reviews and meta-analyses
- Opinion pieces, letter to editors, conference proceedings and presentations, protocols
- Small sample sizes – studies with < 50 cases
- Studies that looked at only one or two pathogens

Appendix III Studies on fever aetiologies in Southeast Asia published from 1st January 2015 to 31st May 2019. Those fully described were considered pivotal.

Reference & Year	Study location	Study setting & Enrolment period	Inpatient – Outpatient	Inclusion criteria	Sample size & Median age	Diagnostic methods	Pathogen distribution
Prodjosoewojo et al., 2019 [161]	Indonesia	Three tertiary-care hospitals & two clinics 2014-2016	Inpatient & Outpatient	≥14-year-old with AUF & suspicion of arbovirosis, salmonellosis, leptospirosis, rickettsiosis	463 47-year-old	Blood culture: BacT/ALERT® (bioMérieux, France). Dengue: Early Rapid & Duo Cassette IgM IgG (Alere, Panbio®, US); Chikungunya: ELISA IgM (in-house CDC Protocol); Arboviral real-time PCR (Bio-Rad, US). *R. typhi*: IgM/IgG & real-time PCR. *Leptospira*: Panbio® IgM ELISA (Alere, US) & PCR; *Salmonella:* Tubex® TF rapid typhoid detection (IDL Biotech AB, Sweden) & PCR. *Plasmodium:* blood smear.	Pathogen detection 190/463 (41%): dengue virus 86 (17.5%); murine typhus 26 (5.6%); *Salmonella* spp. 12 (2.6%); chikungunya virus 8 (1.7%); *Leptospira* 6 (1.3%); *Plasmodium* 4 (0.9%); Dengue virus and bacteraemia 3 (0.7%) including *Salmonella* spp. 2 and *S. aureus* 1
Takeshita et al., 2019 [157]	Vietnam	Tertiary-care hospital 2009-2012	Inpatient	Retrospective blood culture among >15-year-old patients	45,366 NA	Blood culture: BACTEC® 9240 (Becton-Dickinson, USA)	Detection 6,306 (13.9%): coagulase-negative *Staphylococcus* 1,243 (16.7%); *E. coli* 507 (6.8%), *Streptococcus* spp. excluding *S. pneumoniae* 284 (3.8%); *S. aureus* 386 (5.2%); *Klebsiella* spp. 309 (4.2%); *Serratia* spp. 176 (2.4%); *Acinetobacter* spp. 160 (2.2%); *Salmonella*. spp., Typhi or Paratyphi A 137 (0.3%)
Hantrakun et al., 2018 [153]	Thailand	Tertiary-care hospital 2013-2017	Inpatient	≥18-year-old with an infection	4,989 57-year-old	Blood culture: BD BACTEC® (Becton-Dickinson, USA)	Detection 688 (13.8%): *E. coli* 209 (4.2%); *B. pseudomallei* 149 (3.0%); *K. pneumoniae* 38 (0.8%); coagulase-positive *Staphylococcus* 62 (1.2%)
Wangrangsima-kul et al., 2018 [110]	Thailand	Regional hospital 2006-2008	Inpatient	≥15-year-old AUF ≥37.5˚C or a history <21 days & negative malaria blood film	200 41-year-old	Dengue: ELISA PanBio Early NS1, IgM/IgG capture (Alere); Japanese Encephalitis-Dengue: PanBio IgM combo (Alere). *Leptospira*: SD Bioline RDT IgM/IgG and blood culture. Scrub and murine typhus: IgM IFA, culture & PCR (56kDa, 47kDa *htra* and *groEL* genes)	Detection 103 (51.5%): *O. tsutsugamushi* 45 (22.5%); Dengue virus 23 (11.5%); *Leptospira* 15 (7.5%); *R. typhi* 7 (3.5%); BSI 7 (3.5%): T. *marneffei* 2 (1%); *E. coli* 1 (0.5%); *B. pseudomallei* 1 (0.5%); *H. influenzae* 1 (0.5%); *S. aureus* 1 (0.5%); *Salmonella* spp. in stool 1 (0.5%)
Dat et al., 2017 [158]	Vietnam	Tertiary-care hospital 2011-2013	Inpatient	Retrospective cohort of blood cultures	477 48-year-old	Blood culture: Bactec®(Becton Dickinson, USA) with subculture using VITEK and API testing (bioMérieux, France)	*K. pneumoniae* 110 (23.1%); *E. coli* 73 (15.3%); *S. suis* 43 (9.0%); *S. aureus* 41 (8.6%); *S. enterica* 30 (6.3%); *Streptococcus* species 23 (4.8%)
Southeast Asia Infectious Disease Clinical	Indonesia Thailand & Vietnam	13 tertiary-care hospitals 2013-2015	Inpatient	Children ≥30-day-old and adults ≥18-year-old with an infection	1,578 72% under 5-year-old	Blood & urine cultures. Ziehl-Neelsen, sputum culture and multiplex PCR if respiratory symptoms. CSF culture if neurological symptoms. RDT: Dengue (NS1 and IgM, Standard Diagnostics, South Korea),	Detection 553 (35%): Dengue virus 122 (8%); *Leptospira* spp. 95 (6%); rickettsiosis 96 (6%) including *O. tsutsugamushi* 35 (2%); *E. coli* 76

Research Network et al., 2017 [15]					55% [18-59]-year-old	influenza (QuickVue, Quidel Corporation, USA), leptospirosis (IgM/IgG, Standard Diagnostics)	(5%); influenza viruses 65 (4%); Hantaviruses 28 (2%); NTS 21 (1%); *S. aureus* 21 (1%)
Lim et al., 2016 [41]	Thailand	Provincial hospitals 2004-2010	Inpatient	Hospitals who agreed to participate	1,255,571 NA	Blood culture system: Clinical and Laboratory Standards Institute	20,803 positives (1.2%): *E. coli* 4,279 (20.6%); *S. aureus* 1,881 (9%); *K. pneumoniae* 1,661 (8%); *Acinetobacter* spp. 1,065 (5.1%); *P. aeruginosa* 568 (2.7%); *Enterococcus* spp. 342 (0.2%)
Nor Azizah et al., 2016 [162]	Malaysia	Tertiary-care hospital 2001-2011	Inpatient	≥7 days neonates & CAB	222 11.7-month-old	Blood culture: API and VITEK identification system (bioMérieux, France)	*S. aureus* 38 (17.1%); NTS 36 (16.2%); *S. pneumoniae* 28 (12.6%); *E. coli* 14 (6.3%)
Chansamouth et al., 2016 [14]	Lao PDR	Tertiary-care hospital 2006-2010	Inpatient	Pregnant women with tympanic temperature ≥37.5˚C	250 24-year-old	Blood culture (subculture if turbid onto blood and chocolate agar). Urine culture (Oxoid Brilliance UTI Clarity agar). RDT: scrub typhus IgM (AccessBio CareStart); *R. typhi* IgM (ImmunoDot, GenBio, USA). IFA: scrub and murine typhus (Australian Rickettsial Reference Laboratory, Australia). TaqMan real-time PCR: *O. tsutsugamushi* (47 kDa *htrA* gene), *Rickettsia* genus (17 kDa gene), and *R. typhi* (*ompB* gene). Rickettsial culture if positive RDT. *Leptospira*: culture and MAT. Dengue virus and JEV ELISA (PanBio Ltd., Australia). Dengue virus TaqMan real-time RT-PCR (SuperScriptIII Platinum One-Step qRT-PCR system, Invitrogen). HEV ELISA (Beijing Wantai Biological Pharmacy Enterprise Co, China). Malaria smear and Parachek (*Pf*. HRP-2, Orchid Industries, India)	Detection 149 (59.6%): Dengue virus 132 (52.8%); *E. coli* 23 (9.2%); murine typhus 23 (9.2%); scrub typhus 9 (3.6%); *S.* typhi 7 (2.4%); *Leptospira* 1 (0.4%); JEV 1 (0.4%); Dengue virus and scrub typhus 4 (2%); dengue and murine typhus 4 (1.6%); dengue and *E. coli* 4 (1.6%). *M. tuberculosis* 2 (0.8%); *S. aureus* 1 (0.4%); *P. falciparum* 1 (0.4%)
Murni et al., 2016 [160]	Indonesia	Tertiary-care hospital 2010-2013	Inpatient	Children with nosocomial blood stream infections	170 43% under 12-month-old	Blood culture: BACTEC 9120® (BD Diagnostics, Sparks, USA)	*P. aeruginosa* 93 (55%); *K. pneumoniae* 9 (5.3%); *Pseudomonas* spp. 6 (3.5%); *A. baumanii* 2 (1.2%) and *E. coli* 1 (0.6%)
Bhengsri et al., 2016 [154]	Thailand	Six district hospitals 2002-2005	Inpatient & Outpatient	≥7-year-old with AUF >38˚C or history <2 weeks	1,603 23-year-old	IFA for *N. sennetsu, R. honei, R. felis, R. typhi,* and *O. tsutsugamushi* (Gilliam and Kawasaki strains)	*N. sennetsu* 2 (0.1%); SFG 9 (0.6%); MT 35 (2.2%); ST 24 (1.5%)
Prachanukool et al., 2016 [155]	Thailand	Tertiary-care hospital 2014	Inpatient	>15-year-old cancer patients with bacteraemia	775 67-year-old	Blood culture (method not reported)	*S. aureus* 193 (24.9%); *E. coli* 158 (20.4%); *Streptococcus* spp. 88 (11.4%); *K. pneumoniae* 41 (5.3%)
Mayxay et al., 2015 [6]	Lao PDR	Rural district hospital 2003-2004	Inpatient	AUF >37.5˚C or history <21days & negative for malaria	229 15-year-old	Dengue and JEV ELISA kits (Panbio Inc., Australia now Alere Inc., Waltham, MA). Dengue TaqMan RT-PCR. Leptospirosis: MAT. Scrub typhus, murine typhus, and SFG: IFA	Detection 120 (52%): Dengue virus 69 (30.1%); leptospirosis 16 (7.0%); JEV 8 (3.5%); scrub typhus 6 (2.6%); SFG 2 (0.9%); flavivirus 2 (0.9%); murine typhus 1 (0.4%). Dengue virus & leptospirosis 10 (4.4%); murine typhus & SFG 3 (1.3%)
Tran et al., 2015 [159]	Vietnam	Tertiary-care hospital 2010-2011	Inpatient	All new-borns admissions	2,555 NA	Blood culture: incubated in the brain–heart infusion broth at 37˚C for 7 days. If bacteria grew, Gram stains and culture on blood agar and MacConkey agar	Positives in suspected invasive sepsis 106/616 (17.2%): coagulase-negative *Staphylococcus* 23 (3.7%); *Acinetobacter* 17 (2.8%); *K. pneumoniae* 12 (2.0%); *Candida* 24 (3.9%)

Loo et al., 2015 [163]	Singapore	Tertiary-care hospital 2011-2012	Inpatient	≥21-year-old with haemodialysis and vascular access-associated BSI	118 60-year-old	Blood culture (method not reported)	*S. aureus* 68 (57.6%); *Pseudomonas* species 13 (11.0%); *Enterobacter* species 7 (5.9%); *A. baumannii* 5 (4.2%)
Siripaitoon et al., 2015 [156]	Thailand	Tertiary-care hospital 2004-2010	Inpatient	Consecutive ICU patients ≥15-year-old with SLE	61 38.9-year-old	Blood culture (method not reported)	*A. baumannii* 8 (13.1%); *M. tuberculosis* 4 (6.6%); *Aspergillus* spp. 4 (6.6%); *S. aureus* 2 (3.3%); *Streptococcus* spp. 2 (3.3%); *E. coli* 2 (3.3%)
Peremalo et al., 2019							Only one pathogen
Shrestha et al., 2019							Only one pathogen
Dysoley et al., 2019							Only one pathogen
Thanapirom et al., 2019							Microbiological findings not informed
Nguyen et al., 2018							Only two pathogens
Dickson et al., 2018							Only one pathogen
Ocviyanti et al., 2018							No microbiological investigations
Yan et al., 2018							Only two pathogens
Njim et al., 2018							No microbiological investigations
Ng et al., 2018							Only one pathogen
Rermruay et al., 2018							Only one pathogen
Kalimuddin et al., 2018							Only one pathogen
Sattabongkot et al., 2018							Only one pathogen
Lie et al., 2018							Case report
Chien et al., 2018							Only one pathogen
Mather et al., 2018							Only one pathogen
Nivesvivat et al., 2018							Only one pathogen
Imad et al., 2018							No microbiological investigations
Camprubi et al., 2018							No microbiological investigations
de Vries et al., 2018							Travellers
Scinto et al., 2018							Only one pathogen
Darmawan et al., 2018							Only one pathogen
Luk-In et al., 2018							Only one pathogen
Gonzalez et al., 2018							Only one pathogen
Lau et al., 2018							Respiratory infections only
Anderson et al., 2018							Only one pathogen
Owattanapanich et al., 2018							Only one pathogen
Bula-Rudas et al., 2018							Case report
Sit et al., 2018							Only one pathogen
Kampitak et al., 2018							Travellers
Toan et al., 2018							Study protocol
Porter et al., 2018							Only one pathogen

Huggan et al., 2018	Only one pathogen
Tat Trung et al., 2018	No microbiological investigations
Woods et al., 2018	Only one pathogen
Traisathit et al., 2018	Only one pathogen
Boyd et al., 2018	Travellers
Brehm et al., 2018	Travellers
Sa-Ngamuang et al., 2018	Only one pathogen
Koh et al., 2018	Only one pathogen
Wongchidwan et al., 2018	Only one pathogen
Greiner et al., 2018	Only one pathogen
Kuijpers et al., 2017	Only one pathogen
Hills et al., 2017	Only one pathogen
Kheang et al., 2017	Only one pathogen
Schully et al., 2017	Only one pathogen
Barber et al., 2017	Only two pathogens
Pintye et al., 2017	Only one pathogen
Sherley et al., 2017	Case report
Ho et al., 2017	Only two pathogens
Shah et al., 2017	Only one pathogen
Chu et al., 2017	Case report
Teerawattanasook et al., 2017	Microbiological findings not informed
Thein et al., 2017	Under 50 cases
Dat et al., 2017	No microbiological investigations
Moradigaravand et al., 2017	Only one pathogen
Whitehorn et al., 2017	Only one pathogen
Krein et al., 2017	No microbiological investigations
Nikam et al., 2017	Microbiological findings not informed
Sit et al., 2017	Only one pathogen
Suwarto et al., 2017	Only one pathogen
Teparrukul et al., 2017	Only one pathogen
Singhatiraj et al., 2017	Case report
Srisutham et al., 2017	Only one pathogen
Myat et al., 2017	Only one pathogen
Tai et al., 2017	Travellers
Zaw et al., 2017	Only one pathogen
Ferstl et al., 2017	Case report
Tantisiriwat et al., 2017	Antibiotic testing
Chao et al., 2017	Only one pathogen
Sari et al., 2017	Only one pathogen

Kotepui et al., 2017	Only two pathogens
West et al., 2017	Only one pathogen
Thuy-Nhien et al., 2017	Only one pathogen
Cumming et al., 2017	Only one pathogen
Prasetyani et al., 2017	Only one pathogen
Vinnemeier et al., 2017	Travellers
Chantratita et al., 2017	Only one pathogen
Kanjanabuch et al., 2017	Only one pathogen
Md Ralib et al., 2017	No microbiological investigations
Katanami et al., 2017	Case report
Phikulsod et al., 2017	Only one pathogen
Kingsley et al., 2016	Only one pathogen
Ng et al., 2016	Only one pathogen
Tun et al., 2016	No microbiological investigations
Kurniawan et al., 2016	No microbiological investigations
Xu et al., 2016	Only one pathogen
Mursinah et al., 2016	Only one pathogen
Alirol et al., 2016	Symposium
Pan et al., 2016	No microbiological investigations
Pava et al., 2016	Only one pathogen
Wood et al., 2016	No microbiological investigations
Tan et al., 2016	Only one pathogen
Un et al., 2016	No microbiological investigations
Tongyoo et al., 2016	Microbiological investigations not
Lubell et al., 2016	Findings reported previously
Prakit et al., 2016	Only one pathogen
Chantratita et al., 2016	Only one pathogen
Ngwe Tun et al., 2016	Only one pathogen
Burns et al., 2016	Case report
Janewongwirot et al., 2016	Only one pathogen
Phan et al., 2016	Only one pathogen
Wiyatno et al., 2016	Only one pathogen
Wacharasint et al., 2016	Microbiological investigations not
Thipmontree et al., 2016	Only one pathogen
Pooripussarakul et al., 2016	No microbiological investigations
Zueter et al., 2016	Only one pathogen
Yung et al., 2016	Only one pathogen
Peto et al., 2016	Only one pathogen
Lawtongkum et al., 2016	Only one pathogen

Chatproedprai et al., 2016	No microbiological investigations
Robb et al., 2016	Only one pathogen
Simarmata et al., 2016	Only one pathogen
Domthong et al., 2016	Only one pathogen
Peto et al., 2016	Only one pathogen
Wilairat et al., 2016	Only one pathogen
Pyke et al., 2016	Only one pathogen
Tavakolipoor et al., 2016	Travellers
Hearn et al., 2016	Only one pathogen
Phommasone et al., 2016	No microbiological investigations
Heah et al., 2016	No microbiological investigations
Owatanapanich et al., 2016	Only one pathogen
Sethaphanich et al., 2016	Only two pathogens
Zavattoni et al., 2016	Travellers
Hwee et al., 2016	Microbiological investigations not
Shinohara et al., 2016	Only one pathogen
Trojanek et al., 2016	Travellers
Hahn et al., 2016	Only one pathogen
Imwong et al., 2016	Only one pathogen
Riswari et al., 2016	Only one pathogen
van Bruggen et al., 2016	Only one pathogen
Moïsi et al., 2016	Only one pathogen
Lau et al., 2016	Only one pathogen
Fujiko et al., 2016	Only one pathogen
Leung et al., 2015	Case report
Downs et al., 2015	Case report
Tan et al., 2015	Only one pathogen
Fortuna et al., 2015	No microbiological investigations
Huu et al., 2015	No microbiological investigations
Villanueva et al., 2015	Only one pathogen
Abdul-Aziz et al., 2015	Antibiotic study
Lubell et al., 2015	Findings reported elsewhere
Dan et al., 2015	Travellers
Hamaguchi et al., 2015	Only two pathogens
Vongbhavit et al., 2015	Only one pathogen
Carter et al., 2015	Only one pathogen
Hatrongjit et al., 2015	Case report
Wang et al., 2015	Only one pathogen
Miyata et al., 2015	Only one pathogen

de Mast et al., 2015	Only one pathogen
Khongwichit et al., 2015	Under 50 cases
Karyana et al., 2015	No microbiological investigations
Cucunazangsih et al., 2015	Only one pathogen
Dittrich et al., 2015	Only one pathogen
Gharib et al., 2015	Case report
Jiang et al., 2015	Only one pathogen
Ong et al., 2015	No microbiological investigations
Laochan et al., 2015	Only one pathogen
White et al., 2015	Only one pathogen
Ho et al., 2015	Only respiratory presentation
Kotepui et al., 2015	Only one pathogen
Whitehorn et al., 2015	Only one pathogen
Yong et al., 2015	Only one pathogen
Foong et al., 2015	Only one pathogen
Fong et al., 2015	Only one pathogen
Tansiri et al., 2015	Only one pathogen
Hsu et al., 2015	Only one pathogen
Bruhn et al., 2015	Only one pathogen
Vlieghe et al., 2015	Only one pathogen
Thong et al., 2015	Only one pathogen
Keeratichananont et al., 2015	No microbiological investigations
Liu et al., 2015	Only one pathogen
Canier et al., 2015	Only one pathogen
Soni et al., 2015	Case report
Boo et al., 2015	Only one pathogen
Tojo et al., 2015	Only one pathogen
Reynolds et al., 2015	Travellers
Lai et al., 2015	Only respiratory presentation
Kawasaki et al., 2015	No microbiological investigations
Thant et al., 2015	Only one pathogen

AF: attributable fraction; API: analytical profile index; AUF: acute undifferentiated fever; BSI: blood stream infection; CAB: community-acquired bacteraemia; ED: emergency department; ELISA: enzyme-linked immunosorbent assay; GNB: gram-negative bacteria; HAB: health-acquired bacteraemia; Hrp2: histidine-rich protein 2; IFA: immunofluorescence antibody; IMAI: Integrated Management of Adolescent and Adult Illness District Clinician Manual; MAT: microscopic agglutination test; NP swabs: nasopharyngeal swabs; NS1: non-structural protein-1; RDT: rapid diagnostic test; PCR: polymerase chain reaction; PFLSH: *Plasmodium falciparum* lactate dehydrogenase; RT-PCR: Reverse-Transcriptase polymerase chain reaction; SLE: systemic lupus erythematosus; SIRS: systemic inflammatory response syndrome.
A. baumannii: Acinetobacter baumannii; B. pseudomallei: Burkholderia pseudomallei; EBV: Epstein-Barr virus*; E. coli: Escherichia coli;* GAS: group A *Streptococcus;* HHV6: human herpesvirus 6; JEV: Japanese encephalitis virus; *K. pneumoniae: Klebsiella pneumoniae; M. tuberculosis: Mycobacterium tuberculosis; N. sennetsu*: *Neorickettsia sennetsu;* NTS: non-*Salmonella* typhi; *O. tsutsugamushi: Orientia tsutsugamushi; P. falciparum: Plasmodium falciparum; R. honei: Rickettsia. Honei; R. felis: Rickettsia felis; R. typhi: Rickettsia typhi*; RSV: respiratory syncytial virus; *P. aeruginosa: Pseudomonas aeruginosa;* SFG: spotted fever group; *S. aureus: Staphylococcus aureus; S. maltophilia: Streptococcus maltophila; S. pneumoniae: Streptococcus pneumoniae; S. suis: Streptococcus suis; T. marneffei: Talaromyces marneffei.*

APPENDIX IV

Literature review – Search strategy

Query: What is performance of C-reactive protein for distinguishing bacterial from viral pathogens in the tropics?

Search String

(((((("Bacterial Infections"[Mesh]) OR "Virus Diseases"[Mesh]) OR "Zoonoses"[Mesh]) OR "Bacteremia"[Mesh])) AND (("Asia, Southeastern"[Mesh]) OR "Africa South of the Sahara"[Mesh]))) AND (("C-Reactive Protein"[Mesh]) OR C-reactive protein [Title/Abstract]) AND (Humans [Mesh] AND English[lang])

Filters used:

- Published time – no limit
- English language
- Species: humans

Excluded

- No microbiological investigations or not informed
- Opinion pieces, letter to editors, conference proceedings and presentations, protocols
- Small sample sizes – studies with < 50 cases

Appendix IV Studies on C-reactive protein levels and performance in distinguishing bacterial from viral pathogens in Southeast Asia and sub-Saharan Africa published from 1st January 2015 to 31st May 2019. Those fully described were considered pivotal

Reference & Year	Study location	Study setting & Enrolment period	Inpatient – Outpatient	Inclusion criteria	Sample size & Median age	Aetiological investigations	C-reactive protein (CRP) levels (in mg/L) – Performance
Prodjosoe-wojo et al., 2019 [161]	Indonesia	Three tertiary-care hospitals and two clinics 2014-2016	Inpatient & Outpatient	Prospective study ≥14-year-old with AUF & suspicion of arbovirosis, salmonellosis, leptospirosis, rickettsiosis	463 47-year-old	Blood culture: BacT/ALERT® with Vitek 2 system (bioMérieux, France). Dengue: Early Rapid and Duo Cassette IgM/IgG (Alere, Panbio®, US); Chikungunya: ELISA IgM (in-house CDC Protocol); Arboviral real-time PCR (Bio-Rad, US); *R. typhi*: IgM/IgG, real-time PCR. *Leptospira*: Panbio® IgM ELISA (Alere, US), PCR; *Salmonella:* Tubex® TF rapid typhoid detection (IDL Biotech AB, Sweden), PCR.	CRP median by aetiology: bacterial 110 (52-192); arboviral 11 (5-23) CRP >20: Se 88.3%, Spe 71.9%, PPV 76.9%, NPV 85.3% CRP >40: Se 84.0%, Spe 91.0%, PPV 90.8%, NPV 84.4%
Bedell et al., 2018 [233]	Malawi	Two district hospitals 2010	Inpatient	Prospective study ≥15-year-old HIV-infected patients with negative sputum smears and history of weight loss or history of fever or diarrhoea >1 month	452 NA	Smears: Lowenstein-Jensen media and fluorescent microscopy (Auramine O), confirmed by Ziehl-Neelsen. Mycobacterial speciation using microscopic cording and MBP-64 lateral flow assays (Capilia®; TAUNS Laboratories, Inc., Japan). Blood culture (BacT/ALERT 3D; bioMerieux® SA, France). Mycobacterial culture (BACTEC Myco/F Lytic®; Becton Dickinson Microbiology Systems, USA).	Confirmed/Probable TB: CRP median 50 (20-120) CRP 3: Se 88%, Spe 33%, PPV 21%, NPV 94% CRP 5: Se 88%, Spe 40%, PPV 22%, NPV 95%; CRP 10: Se 87%, Spe 51%, PPV 25%, NPV 95% CRP 20: Se 79%, Spe 62%, PPV 28%, NPV 94% CRP 50: Se 52%, Spe 79%, PPV 32%, NPV 89% Blood stream infection: CRP median 30 (10-65) CRP 3: Se 82%, Spe 33%, PPV 19%, NPV 91% CRP 5: Se 80%, Spe 40%, PPV 20%, NPV 92% CRP 10: Se 70%, Spe 51%, PPV 21%, NPV 90% CRP 20: Se 60%, Spe 62%, PPV 23%, NPV 89% CRP 50: Se 32%, Spe 79%, PPV22%, NPV 86%
Dat et al., 2018 [208]	Vietnam	Tertiary-care hospital 2011-2013	Inpatient	Retrospective study Positive blood culture <48h of admission	393 48-year-old	Blood culture (method not informed)	CRP median <5: 4.1%; CRP median ≤20: 7.6%; CRP median ≤100: 32.8.5%; CRP median >100: 55.4%
Farr et al., 2018 [216]	Uganda	Tertiary-care hospital 2011-2013	Inpatient	Prospective study HIV-infected patients with cough ≥2 weeks	262 34-year-old	Sputum for AFB and GeneXpert MTB/RIF testing	CRP median: no TB 69 (23-158) *vs* TB 140 (78-209) Se 90%, Spe 33.1%
Wangrang-simakul et al., 2018 [110]	Thailand	Tertiary-care hospital 2006-2008	Inpatient	Retrospective study ≥15-year-old with AUF ≥37.5˚C or a history <21 days & negative malaria on blood film	200 41-year-old	Dengue: ELISA PanBio Early NS1, IgM/IgG capture (Alere); Japanese Encephalitis-Dengue: PanBio IgM combo (Alere). *Leptospira*: SD Bioline RDT IgM/IgG and blood culture. Scrub and murine typhus: IgM IFA, culture and PCR (56kDa, 47kDa *htra* and *groEL* genes)	CRP >20: bacterial 91.7%, viral 27.3%, Se 91.7%, Spe 72.7%, PPV 91.7%, NPV 72.7%, correctly identified 87.2% CRP >40: bacterial 86.1%, viral 13.6%, Se 86.1%, Spe 86.4%, PV 95.4%, NPV 65.5%, correctly identified 86.2% Optimal cut-off CRP 36: Se 88.9%, Spe 86.4%

							Area under ROC curve (95% CI) 0.91 (0.85-0.96)
Ariyanto et al., 2018 [192]	Indonesia	Tertiary-care hospital 2013-2014	Inpatient	Prospective study 18-40-year-old ART-naïve HIV patients	78 32-year-old	CMV: IgG antibody (Sigma- Aldrich, USA), PCR targeting the *UL54* gene	CRP median: CMV DNA+ 1.7 (0.1-17) CRP median: CMV DNA- 2.3 (0.08-139)
Baggett et al., 2017 [185]	Bangladesh, Thailand The Gambia, Kenya, Mali, South Africa & Zambia	Tertiary-care hospital 2011-2014	Inpatient	Prospective case-control study Children 1-59-month-old with severe or very severe pneumonia	56 MCCP and 4,035 non-MCCP NA	NP & OR swabs: multiplex real-time PCR (*lytA* gene) (Applied Biosystems 7500 (ABI-7500) platform); culture & serotyping with Quellung reaction / latex agglutination; pleural fluid: pneumococcal antigen (Binax NOW; Alere); blood culture (only cases, method not described)	CRP ≥40 & high pneumococcal colonisation density increases risk of severe pneumonia CRP ≥40 & non-MCCP: 25% when low pneumococcal density; 44% when high pneumococcal density. CRP ≥40 & MCCP: 84% when low pneumococcal density; 80% when high pneumococcal density
Kuijpers et al., 2017 [194]	Cambodia	Urban referral hospital 2008-2015	Inpatient	Retrospective study Culture-confirmed enteric fever	254 *S.* typhi 23-year-old and *S.* paratyphi A 26-year-old	Blood culture: brain heart infusion broth (BIO-RAD, USA) (2007–March 2009) and from April 2009 onward BacT/ALERT (bioMérieux, France), serotype using commercial antisera (Sifin, Germany) and the White-Kauffmann-Le Minor scheme, confirmation WGS	CRP median: *S.* typhi 47 (38.6-79.2); *S.* paratyphi A 36.0 (25.2-54.7), p =0.034
Hildenwall et al., 2017 [209]	Tanzania	OPD from a rural district hospital 2011-2012	Outpatient	Prospective study 3-month to 5-year-old febrile non-severe children	428 NA	Blood culture (BacT/ALERT 3D; bioMerieux® SA, France). Urine culture (significant growth $\geq 10^5$ colony-forming units)	CRP median: no bacterial infection 7; urine culture positive 24; blood culture positive 22. AUC: urine culture 0.62 (0.50-0.74); blood culture 0.60 (0.34-0.86). CRP median all illness 19; Se 44.6%, Spe 78.5%, PPV 35.9%, NPV 84.0%; UTI: Se 57.1%, Spe 78.4%, PPV 17.9%, NPV 95.7%; bacteraemia: Se 50.0%, Spe 78.5%, PPV 3.3%, NPV 99.1%
Evans et al., 2017 [223]	Zimbabwe	Urban clinics 1997-2001	Outpatient	Prospective study Neonates born to HIV-positive mothers who remained negative ≥ 6-month-old	231 HEU & 100 HIV-unexposed neonates Mothers: 23-year-old	CMV: real-time PCR (Abbott m200rt platform, USA)	CRP median: HEU 0.43 (0.17-1.23); HIV-unexposed 0.20 (0.09-0.48), p <.001. HIV-unexposed: CMV-positive 0.19 (0.10-0.48), CMV-negative 0.21 (0.05-0.80). HEU: CMV-positive 0.49 (0.21-1.42), CMV-negative 0.30 (0.11-0.66), p =0.008
Blake et al., 2017 [226]	Togo	Five district hospitals 2010-2013	Inpatient	Prospective study Clinical suspicion of pneumonia	1,501 30% under 14-year-old	Blood culture (BacT/ALERT 3D; bioMerieux® SA, France). NP aspirates: multiplex PCR (Fast Track Diagnostics, Luxembourg). CXR	CRP ≥40: 59.0% Likely pneumococcal pneumonia: CRP >71.2, Se 77.7%, Spe 77.2% (latent class analysis)
Peltola et al., 2016 [224]	Latin America & Angola	Hospital 1996-2003 (LatAm) and 2005-2007	Inpatient	Prospective study 2-month to 16 (LatAm) or 13 (Angola)-year-old & bacterial meningitis	285 (Lat Am) & 384 (Angola) NA	CSF culture, PCR and antigen detection	CRP median: *S. pneumoniae* 160 (both LatAm & Angola) *H. influenzae* 160 (LatAm) and 161 (Angola) *N. meningitis* 189 (LatAm) and 134 (Angola).
Mendy et al., 2016 [215]	The Gambia	TB clinic	Outpatient	Prospective study Newly diagnosed smear-positive TB	91 29-year-old	Sputum: AFB by Ziehl-Neelsen stain and culture in liquid media (BACTEC™ MGIT™ BD, USA).	CRP median at enrolment 108 (64-158)
Elfving et al., 2016 [210]	Zanzibar	Rural primary health centre 2011	Outpatient	Prospective study	677 cases 14-month-old	Blood: PCR (DBS) on *Plasmodium*, *Rickettsia* spp. (*gltA*, *bioB* & *orfB* genes), dengue, chikungunya, WNV and RVFV.	CXR-confirmed pneumonia CRP 61.5 *vs* No CXR-confirmed pneumonia 26.0 (p <.001)

				2-59-month-old with axillary T ≥37.5˚C and/or history <24h	167 controls	NP and rectal swabs: qPCR. Urine culture and *S. pneumoniae* RDT	
Drain et al., 2016 [187]	South Africa	Tertiary-care hospital 2008-2009	Outpatient	Prospective study ≥18-year-old with AFB-smear negative and suspicion of TB	90 36-year-old	Sputum smear microscopy (Ziehl-Neelsen and Auramine stains) for AFB, culture in liquid media (BACTEC™ MGITTM™ BD, USA). Urine LAM assay (Determine™) TB LAM test (Alere Inc., USA)	CRP mean in TB culture positive: 77.3 *vs* TB culture negative 41.8 CRP ≥25, AUC (95% CI): sputum AFB smear positive or urine LAM positive 0.71 (0.58-0.83)
Jacobs et al., 2016 [188]	South Africa	Primary care clinic 2010-2012	Outpatient	Prospective study ≥18-year-old with cough ≥2 weeks and suspicion of TB	104 38-year-old	Saliva: culture (MGIT method, BD Biosciences) & AFB (Ziehl-Neelsen method and Capilia TB testing, TAUNS, Japan)	CRP median: TB cases 84.7 (46.8-128.1) *vs* no TB cases 31.9 (22-45.5) CRP cut-off 53: Se 75%, Spe 85%AUC 0.83 (0.73-0.94)
Hunt et al., 2015 [217]	Sierra Leone	Ebola treatment centre 2014-2015	Inpatient	Prospective study Ebola-confirmed patients	150 26-year-old	RT-PCR assays for Ebola (Altona, Hamburg, Germany)	CRP median: survivors 12.9 (5.0-29.4); died 69.5 (22.5-147.0); total 21.4 (6-77)
Lubell et al., 2015 [107]	Cambodia, Lao PDR & Thailand	Rural hospitals and clinics 2008-2013	Inpatient & Outpatient	Retrospective study <16-year-old with axillary T ≥38˚C; 5-49-year-old with tympanic T ≥38˚C and no obvious causes; ≥5-year-old with T ≥38˚C and no obvious cause	1,372 NA	Blood culture; PCR; ELISA (3 studies combining different diagnostic tools)	Dengue virus: CRP 10: 46%; CRP 20: 28% JEV: CRP 10: 64%; CRP 20: 52%; Influenza viruses: CRP 10: 49%; CRP 20: 29%; Rickettsial infections: CRP 10: 94%; CRP 20: 80%; *Leptospira*: CRP 10: 97%; CRP 20: 92%; bacteraemia: CRP 10: 93%; CRP 20: 86%; CRP 10: Se 95%, Spe 49%; CRP 20: Se 86%, Spe 67% AUC 0.83 (0.81-0.86)
Huang et al., 2014 [225]	The Gambia	District hospital 2007-2010	Inpatient	Retrospective study 2-59-month-old with pneumonia	202 cases 15-year-old 186 controls	Blood culture (BACTEC 9050® system (BD, New Jersey, USA)	CRP: controls 6.19; mild pneumonia 93.1; severe pneumonia 258; very severe pneumonia 116. Pneumonia: probable bacterial 283 *versus* probable non-bacterial 175 AUC: 0.68 (0.60-0.76)
Mkony et al., 2014 [227]	Tanzania	Tertiary-care hospital 2012-2013	Inpatient	Prospective study Non-severe neonates with septicaemia	208 5.6-day-old	Blood culture: BacT/Alert PF® (Organon-Teknika Corp., USA). Blood, MacConkey and chocolate agars (Oxoid, UK). Identification on microscopic and colonial characteristics; VITEX and API 20E (BioMerieux, France).	CRP cut-off 5: Admission CRP: Se 87.5%, Spe 70.8%, PPV 41.7%, NPV 95.9%, AUC (95% CI) 0.86 (0.78-0.93) Second CRP: Se 78.9%, Spe 75.8%, PPV 42.3%, NPV 93.9%, AUC 0.88 (0.80-0.95)
Mueller et al., 2014 [21]	Cambodia	Three rural health centres 2008-2010	Outpatient	Prospective study 7-49-year-old non-severe with tympanic T >38˚C and <8 days	1,193 23-year-old 282 non-febrile 31-year-old	Blood culture (Pharmaceutical Factory No. 2, Lao PDR). Nested-PCR: *Leptospira* (16srRNA gene), *O. tsutsugamushi* (47 kDa gene), *Rickettsia* (*gltA* and *ompB* genes). RT-PCR: dengue, influenza (throat swabs)	CRP median: Febrile patients 25.7 *vs* non-febrile 1.4 Dengue virus 8.2; influenza virus 9.6; *Leptospira* spp. 19.2; *O. tsutsugamushi* 26.4
Wang et al., 2014 [222]	China and Vietnam	Tertiary-care hospital 2003-2012; 2003-2009; 2013	Inpatient	Retrospective study Influenza-confirmed	133 H7N9 63-year-old 118 H5N1 26-year-old	Virus isolation or real-time RT-PCR; or ≥4 rise antibody titre of an acute serum	CRP median: H7N9 65 (25-113); H5N1 51 (14-118)
Drain et al., 2014 [124]	South Africa	Tertiary-care hospital 2009-2010	Inpatient	Prospective study ≥18-year-old HIV-infected patients with	93 35-year-old	Blood culture (BACTEC® MGITTM® BD, USA) and solid culture media. Positive	CRP mean 70.7, AUC 0.87 (0.79-0.95) CRP cut-off 8: Se 97.4%, Spe 53.5%, PPV 65.5%, NPV 95.8% CRP cut-off 25:

				cough ≥2 weeks and AFB-smear negative		cultures were identified as *M. tuberculosis* using a niacin test.	Se 92.3%, Spe 62.8%, PPV 69.2%, NPV 90.0% CRP cut-off 50: Se 76.9%, Spe 74.4%, PPV 73.2%, NPV 78.1%
Lawn et al., 2013 [190]	South Africa	ART clinic	Outpatient	Prospective study >18-year-old patients ART naive with trimethoprim-sulphamethoxazole prophylaxis	496 33-year-old	Sputum: fluorescence microscopy, liquid culture (Mycobacterial Growth Indicator Tubes, Becton Dickinson, USA) and Xpert MTB/RIF assay. Urine samples: LAM using Clearview TB-ELISA (Alere Inc., USA) and Xpert MTB/RIF assay	TB-confirmed CRP median 50, AUC 0.81 CRP cut-off 10: Se 85.2%, Spe 57.6%, PPV 28.2%, NPV 95.2% CRP cut-off 20: Se 74.1%, Spe 73.0%, PPV 34.9%, NPV 93.5% CRP cut-off 50: Se 55.6%, Spe 88.7%, PPV 48.9%, NPV 91.1%
Diez-Padrisa et al., 2012 [130]	Mozam-bique	District Hospital 2006-2007	Inpatient	Prospective study <5-year-old with clinical severe pneumonia	586 NA	Blood culture: Bactec® 9050 (Becton Dickinson, USA)	CRP median BC+ 178 *vs* BC- 27; AUC 0.79 CRP cut-off 20: Se 95%, Spe 45%, PPV 22%, NPV 67% CRP cut-off 8: Se 90%, Spe 50%, PPV 23%, NPV 64% CRP cut-off 38: Se 85%, Spe 57%, PPV 24%, NPV 63%
Iwalokun et al., 2012 [220]	Nigeria	Four hospitals 2009-2010	Inpatient	Prospective study 5-25-year-old & SCA	64 cases 16-year-old 41 controls	Parvovirus B19: solid-phase IgM ELISA (Biotrin International, Ireland)	SCA patients, CRP mean PB19 + 8.9 *vs* 5.5 BP19 - Controls, CRP mean PB19 + 2.2 *vs* 1.7 BP19 -
Alvarez et al., 2012 [189]	South Africa	District clinics 2005-2007	Outpatient	Prospective study >18-year-old HIV-infected patients with suspicion of TB and negative smear AFB	228 34-year-old	Sputum AFB: fluorescent microscopy, culture in liquid media (BACTEC™ MGIT™ BD, USA). Positive cultures identified as *M. tuberculosis* using the niacin test	CRP AUC 0.81 (0.76-0.87)
Faurholt-Jepsen et al., 2012 [214]	Tanzania	Urban health centre 2006-2008	Outpatient	Prospective study ≥15-year-old non-severe patients newly diagnosed with TB	1,205 36-year-old	Sputum AFB and culture with Lowenstein Jensen solid media	TB cases with diabetes: CRP median 18.8 (8.2-29.4)
Amah et al., 2011 [218]	Nigeria	Clinic	Outpatient	Prospective study 3-65-year-old with either hepatitis B or malaria	100 cases 40 controls NA	Hepatitis B: HBsAg (Bioline SD)	CRP median hepatitis B 7.1 *vs* malaria 7.8 ($p < 0.05$) CRP median in the controls 3.2
Siau et al., 2011 [221]	Singapore	Tertiary-care hospital 2009	Inpatient	Retrospective study H1N1-confirmed cases	153 26-year-old	NP/ or throat swabs: Gram stain, culture, RT-PCR for H1N1; blood and urine cultures (methods not informed)	CRP median H1N1: mild presentation 27.3; moderate severity 67.2; severe presentation 87.5 ($p = 0.003$)
Rasmussen et al., 2011 [213]	Guinea-Bissau	Three health centres & one tertiary-care hospital 2003-2006	Inpatient & Outpatient	Prospective study ≥15-year-old with TB on smear microscopy	218 cases 29-year-old 50 controls	NA	Low severity: CRP median 39.5 High severity: CRP median 57.1 Controls: CRP median 2.5
Wilson et al., 2011 [123]	South Africa	District clinics 2005-2007	Outpatient	Prospective study >18-year-old HIV-infected patients with suspicion of TB and negative smear AFB	364 34.4-year-old	Sputum AFB: fluorescent microscopy, culture in liquid media (BACTEC™ MGIT™ BD, USA). Positive cultures identification using the niacin test	CRP median TB-confirmed 15.4 *vs* 0.76 in TB-negative Pulmonary TB 12.4 (7.1-17.4); pulmonary and extrapulmonary TB 18.2 (12.6-27.3); extrapulmonary TB 21.6 (9.3-27.6). Se 98%, Spe 59%, PPV 74%, NPV 96% AUC 0.91 (0.87-0.95)

Diez-Padrisa et al., 2010 [129]	Mozam-bique	Rural district hospital 2006-2007	Inpatient	Prospective study <5-year-old with severe pneumonia	190 45% under 1-year-old	Blood culture: Bactec® 9050 (Becton Dickinson, USA).NP aspirates: PCR for RSV, Flu, PIV, hMPV and adenovirus	CRP median: controls 4, viral aetiology 18.3, bacterial aetiology 185.4 CRP cut-off 20.9: Se 94.6%, Spe 54.2%; AUC CRP 0.87
Carrol et al., 2009 [128]	Malawi	Tertiary-care hospital 2004-2006	Inpatient	Prospective study 2-month to 16-year-old with suspicion of pneumonia or meningitis	377 cases 2.3-year-old 13 controls	Blood culture (BacT/ALERT 3D; bioMerieux® SA, France). CSF: culture brain-heart infusion (Oxoid, UK), latex agglutination (Abbott Murex Biotech, UK). Blood/CSF: PCR *N. meningitidis, H. influenzae and S. pneumoniae*	CRP: bacterial meningitis or pneumonia 291 *vs* No bacteria detected 135 CRP: bacterial meningitis or pneumonia except IPD 279 *vs* presence of IPD 294 AUC 0.81 (0.73-0.89)
Wagenaar et al., 2009 [193]	Indonesia	Tertiary-care hospital 2005-2006	Inpatient	Prospective study Clinical suspicion of severe leptospirosis	52 44-year-old	MAT ≥1:320 in a single sample; seroconversion; ≥4-fold increase; ≥1:80 in a single sample from early deceased patient	CRP median 100
Cheung et al., 2008 [212]	The Gambia	Tertiary-care hospital and clinics 2002-2004	Inpatient & Outpatient	Retrospective study 6-51-week-old with fever ≥38˚C and raised respiratory rate or pneumonia	120 with pneumonia 1,868 without	Testing for *S. pneumoniae*: Blood culture: Bactec® 9050 (Becton Dickinson, USA); lung aspirates and CSF: standard microbiological procedures and PCR	CRP median bacteraemia negative around 20 *vs* bacteraemia positive around 180; AUC 0.65 (performance better among inpatients than outpatients) CRP cut-off 40 for pneumococcal bacteraemia: Se 86.7%, Spe 57.0%, PPV 1.7%, NPV 99.8% CRP cut-off 60 for pneumococcal bacteraemia: Se 80.0%, Spe 65.3%, PPV 1.9%, NPV 99.8% CRP cut-off 120 for pneumococcal bacteraemia: Se 73.3%, Spe 80.9%, PPV 3.1%, NPV 99.7%
Tribble et al., 2008 [186]	Thailand	Temporary medical unit	Outpatient	Prospective study ≥18-year-old with acute diarrhoea ≤96h	182 26-year-old	Blood: *Campylobacter* (immunoglobin-secreting cells); stools: *C. jejuni* or *C. coli* using the ProSpecT microplate assay (Alexon-Trend, Inc., USA) and PCR. Rotavirus and calicivirus antigens (Rotazyme; Abbott, USA)	Se 83%, Spe 75%, PPV 56%, NPV 92% AUC 0.81 (0.71-0.91)
Rosales et al., 2002 [454]	Zambia	Urban health centres 1991	Outpatient	Posteriori analysis Non-severe 5-month to 17-year-old with measles	196 35% <12-month-old	Measles hemagglutination inhibition test (HAI): a fourfold increase in titre, from baseline to week 2	CRP median: vitamin A deficient 27; normal vitamin A 11
Choo et al., 2001 [83]	Malaysia	Tertiary-care hospital NA	Inpatient	Retrospective study 1-month to 12-year-old with enteric fever	227 7.3-year-old	Blood and stool cultures Widal and Typhidot tests	CRP median: culture-positive *S.* typhi 43 (12-150) *vs* non-typhoidal illness 21 (4-110) CRP cut-off 30 (culture-positive and negative typhoid *vs* non-typhoidal illness): Se 68%, Spe 58%, AUC 0.64
Michelow et al., 2000 [191]	South Africa	Three urban hospitals 1997	Inpatient	Prospective study Children with meningitis suspicion	113 9-month-old	CSF: Gram stain and culture, viral culture using RT-PCR for enterovirus (Boehringer Mannheim GmbH, Germany)	CRP median: bacterial meningitis 200 (131-200) *vs* viral meningitis 33.5
Sutinen et al., 1998 [82]	Philippines	Tertiary-care hospital 1983-1984	Inpatient	Prospective study Children with meningitis suspicion	103 NA	Blood & CSF cultures: brain-heart infusion broth with SPS and subculture onto blood, chocolate and MacConkey Agar plates. CSF Latex agglutination test, counter-immuno-electrophoresis, viral culture and antibodies	CRP mean: bacterial aetiology (including *S. pneumoniae, H. influenzae* and *N. meningitis*) 207 *vs* viral group 39 CRP cut-off 50: Se 94%, Spe 65%, PPV 57%, NPV 96%
Rosales et al., 1998 [219]	Zambia	Urban health centre	Outpatient	Post-hoc analysis 5-month to 17-year-old with acute measles	200 <60-month-old	Measles haemagglutinin antibody titre	CRP mean: acute measles without vitamin A [5.4-44.7]; acute measles with vitamin A treatment [8.3-61.3]

Mkhize et al., 2018	Evaluation on nutritional status
Harjunmaa et al., 2018	No microbiological investigations
Goovaerts et al., 2018	Less than 50 participants
Galloway et al., 2018	No microbiological investigations
Opolot et al., 2018	No microbiological investigations
Maloupazoa Siawaya et al., 2018	Less than 50 participants
Prendergast et al., 2017	No microbiological investigations
Filteau et al., 2017	No microbiological investigations
Gaziano et al., 2017	No microbiological investigations
PrayGod et al., 2017	No microbiological investigations
Janssen et al., 2017	No microbiological investigations
Borkum et al., 2017	No microbiological investigations
Marbou et al., 2017	Analysis on resistance
Tasher et al., 2017	Case report
Ndlovu et al., 2017	No microbiological investigations
Patel et al., 2017	On one disease progression
Rytter et al., 2017	No microbiological investigations
Ngouleun et al., 2016	On one disease progression
Loots et al., 2016	On one disease progression
Verani et al., 2016	No microbiological investigations
Takele et al., 2016	No microbiological investigations
Brink et al., 2016	No microbiological investigations
Mave et al., 2016	No microbiological investigations
Bipath et al., 2016	No microbiological investigations
Jensen et al., 2016	No microbiological investigations
Hoff et al., 2015	Case report
Phommasone et al., 2016	Rapid tests comparison
Babirekere-Iriso et al., 2016	No microbiological investigations
PrayGod et al., 2016	No microbiological investigations
Olwenyi et al., 2016	On one disease progression
Phatlhane et al., 2016	On one disease progression

White et al., 2016	No microbiological investigations
Mupfudze et al., 2015	No microbiological investigations
Jenkins et al., 2015	Case report
Madhi et al., 2015	No CRP evaluations
James et al., 2015	On one disease progression
Jayakumar et al., 2015	Under 50 participants
Gleason et al., 2015	On one disease progression
Nicholson et al., 2015	No microbiological investigations
Rytter et al., 2015	No microbiological investigations
Fourie et al., 2015	No microbiological investigations
Botha et al., 2015	No microbiological investigations
von Känel et al., 2015	No microbiological investigations
Canipe et al., 2014	No microbiological investigations
Chantong et al., 2014	No microbiological investigations
Tarr et al., 2014	On one disease progression
Hur et al., 2014	On one disease progression
Ssinabulya et al., 2014	On one disease progression
Prendergast et al., 2014	No microbiological investigations
Calvo-Cano et al., 2014	Case report
Faller et al., 2014	On one disease progression
Friis et al., 2013	Metabolic study
Mugisha et al., 2013	No microbiological investigations
McDonald et al., 2013	On one disease progression
Redd et al., 2013	On one disease progression
McDonald et al., 2013	On one disease progression
Ravimohan et al., 2013	On one disease progression
Lawn et al., 2013	On disease severity
Raby et al., 2013	Case report
Kosalaraksa et al., 2012	On one disease progression
Ledwaba et al., 2012	On one disease progression
Kitchin et al., 2012	CRP not evaluated

Bunupur-adah et al., 2012	On one disease progression
Boufassa et al., 2012	On one disease progression
Mullen et al., 2012	On one disease progression
Masaisa et al., 2012	No microbiological investigations
Isanaka et al., 2012	No microbiological investigations
Masaisa et al., 2012	No microbiological investigations
Fourie et al., 2012	No microbiological investigations
Koethe et al., 2011	On one disease progression
Ridruechai et al., 2011	On one disease progression
du Toit et al., 2011	On one disease progression
Conradie et al., 2011	On one disease progression
Fourie et al., 2011	No microbiological investigations
Haddow et al., 2011	On one disease progression
Worodria et al., 2011	On one disease progression
Redd et al., 2010	On one disease progression
Mburu et al., 2010	On one disease progression
Pakasi et al., 2010	No microbiological investigations
Heimburger et al., 2010	On one disease progression
Fourie et al., 2010	No microbiological investigations
Soepandi et al., 2010	CRP not evaluated
Ghosh et al., 2010	No microbiological investigations
Friis et al., 2009	No microbiological investigations
Fufa et al., 2009	No microbiological investigations
Wander et al., 2009	No microbiological investigations
Ho et al., 2009	No microbiological investigations
Calmy et al., 2009	No microbiological investigations
Pakasi et al., 2009	No microbiological investigations
Puumalainen et al., 2008	No microbiological investigations
Zulu et al., 2008	No microbiological investigations
Drain et al., 2007	On one disease progression
Kupka et al., 2007	On one disease progression

Olesen et al., 2007	On one disease progression
Baeten et al., 2007	On one disease progression
Saranchuk et al., 2007	Under 50 cases
Leong et al., 2006	CRP not evaluated
Mairuhu et al., 2005	Under 50 participants
Sirijaichin-gkul et al., 2005	Under 50 cases
Larsson et al., 2005	No microbiological investigations
Arinola et al., 2004	On one disease progression
Liew et al., 2004	No microbiological investigations
Koegelenberg et al., 2004	No microbiological investigations
Visser et al., 2003	On one disease progression
Campbell et al., 2003	No microbiological investigations
Massawe et al., 2002	No microbiological investigations
Baten et al., 2002	No microbiological investigations
Eley et al., 2002	No microbiological investigations
Hinderaker et al., 2002	No microbiological investigations
Semba et al., 2000	No microbiological investigations
Lawn et al., 2000	On one disease progression
Massawe et al.,1999	No microbiological investigations
Lawn et al., 1999	Under 50 cases
Ekanem et al.,1997	Under 50 cases
Garred et al.,1997	No microbiological investigations
Ebbesen et al.,1995	No microbiological investigations
Cuevas et al.,1995	Under 50 cases
Kuvibidila et al.,1991	On one disease progression
Kardjito et al.,1980	Article not available
Mc Farlane et al., 1967	No microbiological investigations

AFB : acid-fast bacilli; API: analytical profile index; AUC: area under the receiver operating characteristic curve; BC: blood culture; CNF: cerebrospinal fluid; CNS: central nervous system; CXR: chest X-ray; DBS: dried blood spot; ED: emergency department; ELISA: enzyme-linked immunosorbent assay; HEU: HIV-exposed uninfected; IFA: immunofluorescence antibody; IPD: invasive pneumococcal disease; LAM: Lipoarabinomannan; LatAm: Latin America; MAT: microscopic agglutination test; MCCP: microbiologically confirmed pneumococcal pneumonia; MGIT: mycobacterial growth indicator tubes; NP: nasopharyngeal; OPD: outpatient department; PCR: polymerase chain reaction; RT-PCR: Reverse-Transcriptase polymerase chain reaction; SPS: sodium polyanethol sulfonate; T: temperature; TB: tuberculosis; WGS: whole genome sequencing.
C. coli: Campylobacter coli; C. jejuni: *Campylobacter jejuni*; JEV: Japanese encephalitis virus; *K. pneumoniae: Klebsiella pneumoniae; M. tuberculosis: Mycobacterium tuberculosis; N. sennetsu*: *Neorickettsia sennetsu;* NTS: non-*Salmonella* typhi; *O. tsutsugamushi: Orientia tsutsugamushi;* PB19: parvovirus B19; *P. falciparum: Plasmodium falciparum; R. honei: Rickettsia. Honei; R. felis: Rickettsia felis; R. typhi: Rickettsia typhi*; RSV: respiratory syncytial virus; *P. aeruginosa: Pseudomonas aeruginosa;* SFG: spotted fever group; *S. aureus: Staphylococcus aureus; S. maltophilia: Streptococcus maltophila; S. pneumoniae: Streptococcus pneumoniae*

APPENDIX V

Literature review – Search strategy

Query: What is the impact of C-reactive protein-testing on antibiotic prescription in primary care?

Search String

(((((("Outpatients"[Mesh] OR "Outpatient Clinics, Hospital"[Mesh] OR "Ambulatory Care"[Mesh] OR "Ambulatory Care Facilities"[Mesh]) OR ("Primary Health Care"[Mesh] OR "Primary Care Nursing"[Mesh] OR "Physicians, Primary Care"[Mesh])) OR "Mobile Health Units"[Mesh])) AND (("C-Reactive Protein"[Mesh]) OR C-reactive protein[Title/Abstract])) AND ((("Anti-Bacterial Agents"[Mesh]) OR antibiotic prescription[Title/Abstract]) OR antibacterial prescription[Title/Abstract])

Filters used

- English Language
- Species: humans

Excluded

- Qualitative studies
- Cost-effectiveness analysis
- Case report or under 50 participants
- Reviews, meta-analyses
- Opinion pieces, letter to editors, conference proceedings and presentations, protocols

Appendix V Studies on the impact of C-reactive protein-testing on antibiotic prescription among febrile patients attending primary care published until 31st May 2019. Those fully described were considered pivotal

Reference & Year	Study location	Study setting & Enrolment period	Inclusion criteria	Sample size & Median age	Methods	C-reactive protein (CRP) levels (in mg/L) – Antibiotic prescription
Keitel et al., 2019 [211]	Tanzania	9 outpatient clinics 2014-2016	2-59-month-old non-severe children with axillary T ≥37.5˚C and cough	1,726 45% under 12-month-old	Randomised controlled non-inferiority trial CRP-testing: semi-quantitative lateral-flow test (bioNexia CRPplus, bioMérieux®) CRP cut-off 80 mg/L	Antibiotic prescription: ePOCT 2.3% *vs* ALMANACH 40.4% *vs* IMCI 44%. Safety: RR 0.30 (0.10-0.93)
Althaus et al., 2019 [100]	Myanmar & Thailand	9 outpatient clinics and 1 OPD district hospital 2016-2017	≥1-year-old non-severe patients with tympanic T >37.5˚C or history of fever <2 weeks	2,410 Children 4-year-old; Adults 34-year-old	Randomised controlled trial. CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) with qualitative information CRP cut-off 20 or 40 mg/L	CRP median 9 (8-22) CRP 40: 30.6% (aOR 0.75 [0.60-0.92]) *vs* CRP 20: 33.5% (aOR 0.86 [0.70-1.06]) *vs* control 36.8%. Safety: HR cut-off 20 after14 days: 0.97 (0.88-1.08); HR cut-off 40: 0.98 (0.88-1.08)
Schot et al., 2018 [282]	The Netherlands	28 general practice 2013-2016	3-month to 12-year-old children with acute cough with suspicion of non-severe LRTI	309 3-year-old	Pragmatic open randomised controlled trial CRP-testing: quantitative Afinion® POCT (Alere Technologies AS, Norway) CRP cut-offs 10 and 100 mg/L	30.9% URTI, 21.3% bronchitis, 13.3% pneumonia. 51% with CRP <10; 4% with CRP >100; mean CRP 22.5. Antibiotic prescription: CRP-testing 30.9% *vs* no CRP-testing 39.4%, OR 0.6 (0.30-1.24). Safety: re-consultations OR 0.95 (0.46-1.99)
Schuijt et al., 2018 [455]	The Netherlands	17 primary care centres 2013-2015	>18-year-old with acute cough and diverticulitis	1,756 NA	Prospective observational study Quantitative CRP POCT (Afinion® AS100 Analyser, USA); CRP-testing if intermediate risk. Antibiotic recommendation: no if CRP <20; no advice if CRP 20-100; yes, if CRP>100 mg/L	65.3% with CRP <20; 28.4% with CRP [20-100]; 6.3% with CRP >100 Antibiotic prescription 3.9% if CRP <20; 69.7% if CRP [20-100]. No antibiotic 4.8% if CRP >100
Lemiengre et al., 2018 [283]	Belgium	133 family physicians 2013-2014	1-month to 16-year-old non-severe children with <5 days infection	2,844 4.6-year-old	Cluster randomised controlled trial. Quantitative CRP POCT (Afinion® AS100 Analyser, USA) CRP cut-off 5 mg/L	CRP mean 20.3 CRP testing *vs* no CRP-testing by EBM guidelines: reduction antibiotic aOR 0.54 (0.33-0.90) CRP <5: no advice aOR 0.24 (0.11-0.50); advice to withhold antibiotic 0.31 (0.17-0.57) CRP more elevated when EBM guidelines advised antibiotics (77.6% *vs* 52.9%, p <.001)
Lemiengre et al., 2018 [284]	Belgium	131 family physicians from 78 practices 2013-2014	1-month to 16-year-old non-severe children with <5 days infection	2,227 5-year-old	Cluster randomised factorial trial Quantitative CRP POCT (Afinion® AS100 Analyser, USA) No CRP guidance	URTI 34.4%, AOM 15.5%, other viral disease 11.8%. Antibiotic prescription: CRP without guidance *vs* usual care aOR 1.01 (0.57-1.79); BISNA *vs* usual care aOR 2.04 (1.19-3.50); CRP & BISNA *vs* usual care aOR 1.17 (0.66-2.09)

Keitel et al., 2017 [99]	Tanzania	9 outpatient clinics 2014-2016	2-59-month-old non-severe children with axillary T ≥37.5˚C and cough	3,192 13-month-old	Randomised controlled non-inferiority trial CRP-testing: semi-quantitative lateral-flow test (bioNexia CRPplus, bioMérieux®) CRP cut-off 80 mg/L	Antibiotic prescription: e-POCT 11.5% *vs* ALMANACH 29.7% (p <.001) *vs* usual care 94.9% Safety: clinical failure e-POCT 2.3% *vs* ALMANACH 4.1% (RD -1.7 [-3.0, -0.5])
van den Bruel et al., 2016 [285]	UK	Two primary care practices 2013-2014	1-month to 16-year-old non-severe children with illness ≤5 days	200 2.8-year-old	Prospective cohort study nested in an individually randomised controlled trial CRP-testing: CRP POCT (Afinion® AS100 Analyser, USA); CRP cut-offs 20 and 80 mg/L	54 patients in the nested RCT. CRP median 21, 5% with CRP >80 Antibiotic prescription: CRP-testing 38.5% *vs* no CRP-testing 32.1% (p =0.627), during 10-day follow-up 35.3% *vs* 5.3% (p =0.023).
Do et al., 2016 [274]	Vietnam	10 primary care centres 2014-2015	≥1-year-old with non-severe acute respiratory infection	2,037 CRP: 16-year-old; Control 15-year-old	Randomised controlled trial CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-off 10 for 1-5-year-old or 40 mg/L for >6-year-old	Antibiotic prescription CRP 64.4% *vs* controls 77.9%; aOR 0.49 (0.40-0.61). Safety analysis: similar time to resolution of symptoms and hospitalisations
Rebnord et al., 2016 [286]	Norway	4 primary care & 1 emergency clinic 2013-2015	0-6-year-old with fever or respiratory symptoms	397 2.3-year-old	Randomised controlled observational study CRP-testing method not informed	Antibiotic prescription: pre-test with CRP 26% *vs* control group 22% (p ≥.05)
Yebyo et al., 2016 [456]	Ethiopia	4 rural primary health centres NA	≥18-year-old with <21 days URTI or pneumonia or acute bronchiolitis	414 40.3-year-old	Cross-sectional registration study CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-off 20, 20-99 and 100 mg/L	Antibiotic prescription 87.8% CRP median 11 (8-29). 66.6% with CRP <20; 27.9% with CRP 20-99; 5.5% with CRP >100
Minnaard et al., 2016 [277]	The Netherlands	9 primary care centres 2012	≥18-year-old with cough <24 hours	1,473 eligible 348 recruited 48-year-old	Retrospective observational study Quantitative CRP POCT (Afinion® AS100 Analyser, USA); CRP cut-off 20 mg/L	CRP-testing: recruited patients 81% *vs* non-recruited 6%. Antibiotic prescription: recruited 44% *vs* non-recruited 26%
Hughes et al., 2016 [300]	UK	One general practice surgery 2015-2016	≥18-year-old with LRTI ≥12 hours and antibiotic decision unclear	94 49.4-year-old	Prospective observational before-after trial Quantitative CRP POCT (Afinion® AS100 Analyser, USA); CRP cut-off 20 (withhold antibiotics, 20-100 (delayed antibiotics), >100 mg/L (antibiotics recommended)	Among 94 patients, 71 with respiratory symptoms. Antibiotic prescription 25.4% with CRP-testing *vs* 46.8% no testing (p =0.04). No delayed prescription when CRP 20-100, 92.7% with CRP <30 without antibiotics. 77.5% with CRP <20.
Salwan et al., 2015 [457]	Norway	7 general practices 2009-2010	>40-year-old with a diagnosis of asthma or COPD	95 62.1-year-old	Observational multicentre prospective study CRP-testing: Afinion® AS100 Analyzer (Axis-Shield, Scotland), Orion Quickread CRP (Orion Diagnostica, Finland), ABX Micros CRP (HORIBA medical, France)	Antibiotic prescription: CRP <8: 14.6%; CRP 8-39: 38.1%; CRP ≥40: 71.4%. Predictor for antibiotic: CRP ≥8: aOR 4.3 (1.3-14.8), p =0.02.
Andreeva et al., 2014 [113]	Russia	8 general practices 2010	≥18-year-old with cough/LRTI <28 days	179 50.8-year-old	Open cluster randomised trial CRP-testing: Afinion® test system (Axis Shield). CRP cut-offs 20, 20-50 and ≥50 mg/L	Mean CRP 11.5; in URTI all <50. Antibiotic prescription: CRP group 37.6% *vs* control group 58.9% (p =0.006); 85% if CRP >20; 28% if CRP <20. Safety: recovery after 2 weeks similar in two groups

Llor et al., 2014 [458]	Spain	9 communities 2008-2009	All participants with an ARI	281 general practitioners 27,833 ARIs	Before-after quality assurance study CRP method not informed	Antibiotic prescription: pre-intervention 27.7%; CRP <10: 9%, CRP 11-20: 50%, CRP >20: 80%. OR full intervention (CRP POCT): common cold 0.03 (0.01-0.06); influenza 0.01 (0-0.07); acute pharyngitis 0.15 (0.09-0.25); acute bronchiolitis 0.31 (0.20-0.47)
Cals et al., 2013 [117]	The Netherlands	20 family practices 2005-2010	>18-year-old with suspected LRTI and cough <4 weeks	379 ~ 50-year-old	Pragmatic cluster randomised factorial controlled trial with a 3.5 years follow-up CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway). CRP cut-offs 20 and 100 mg/L	Episodes of ARI: communication group 0.36 PPPY *vs* no training group 0.57 PPPY (p =.09). Antibiotic prescription: CRP-testing all patients 31.5% *vs* no testing 54.5% (p<.01); ARI: CRP-testing 30.7% *vs* no CRP-testing 35.7% (p =0.36); training communication 26.3% *vs* no training 39.1% (p =.02)
Little et al., 2013 [119]	Belgium, Spain, UK, Poland & The Netherlands	246 practices audited 2011	>18-year-old with URTI or LRTI without antibiotic use in the previous month	6,771 49.6-year-old	Multinational cluster randomised factorial controlled trial CRP-testing: QuikRead CRP kits (Orion Diagnostica, Finland); CRP cut-offs ≤20 (withhold antibiotics), 21-50 (withhold antibiotics), 51-99 (withhold or delayed antibiotics), ≥100 mg/L (antibiotics)	LRTI 80% *vs* URTI 20%. Antibiotic prescription: no CRP training 48% *versus* training 33%, RR 0.54 (0.42-0.69); communication: no training 45% *vs* training 36%, RR 0.69 (0.54-0.87). Safety: no CRP training *vs* training RR 1.05 (0.78-1.39); communication: training *vs* no training RR 1.33 (0.99-1.74) Combination CRP-Communication RR 0.38 (0.25-0.55)
Peters et al., 2013 [299]	The Netherlands	4 primary care centres 2010-2011	All patients with intellectual disabilities and suspicion of LRTI	192 49.7-year-old	Prospective case-control study CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-off 40 mg/L	CRP mean if antibiotic prescribed: 106.8 Antibiotic prescription: CRP 41% *vs* no CRP 90%; OR 12.0 (4.1-35.3). Safety: no difference in outcome after 1 month
Hoffmann et al., 2013 [278]	Austria	30 general practitioners 2008-2011	All patients with a respiratory tract infection	677 ~ 42-year-old	Retrospective cross-sectional study CRP-testing: semi-quantitative CRP test (Schwerin, Diagnostik Nord, Germany) CRP cut-off 10 and 30 mg/L	CRP<10: 41%, antibiotic prescription 9.2%; CRP 10-30: 35.5%, antibiotic prescription 71.7%; CRP >30: 21.9%, antibiotic prescription 98.7% 36% with a CRP >30 & prescription did not fill it. 14.4% with CRP <10 and 20.0% with CRP 10-30 took an antibiotic without prescription
Llor et al., 2013 [270]	Denmark, Sweden, Lithuania Russia, Spain & Argentina	617 general practitioners 2008	Patients with AECOPD	1,233 64.5-year-old	Prospective cross-sectional study CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-offs 20 and 100 mg/L	Antibiotic prescription 78.7%; no CRP-testing 80.6%; CRP-testing 54.3%; aOR 0.34 (0.19-0.61)
Llor et al., 2012 [296]	Spain	340 general practitioners 2008-2009	Patients with rhinosinusitis	836 39.8-year-old	Non-randomised controlled before-after study CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-offs 10 and 40 mg/L	Antibiotic prescription: 56.7% CRP group *vs* 86.7% control group (p <0.001), OR 0.12 (0.01-0.32). CRP <10: OR 0.008 (0-0.015)

Llor et al., 2012 [298]	Spain	268 general practitioners 2008-2009	Patients with LRTI	5,385 LRTIs NA	Prospective non-randomised controlled before-after study CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway) CRP cut-offs 20 and 100 mg/L	Antibiotic prescription: control group 76.6% *vs* CRP-testing 43.9%. CRP <10: 51.2% with prescription 13.8%; CRP 11-20 with prescription 57.1%; CRP >20 with prescription 78.9%
Kavanagh et al., 2011 [301]	Ireland	One primary care practice 2010	≥18-year-old with acute cough and/or sore throat <1 month	120 48-year-old	Pilot cross-sectional study CRP-testing: QuickRead® CRP, Orion Diagnostica, Finland. CRP cut-offs <20 (withhold antibiotics), 20-50 (delayed antibiotics), ≥50 mg/L (antibiotics)	63% URTI *vs* ~30% LRTI. Antibiotic prescription: CRP-testing 45% *vs* no testing 58%; delayed antibiotics CRP-testing 37% *vs* no testing 28%. Safety: re-consultation CRP-testing 25% *vs* no testing 15%
Steurer et al., 2011[459]	Switzerland	86 general practitioners 2006-2009	≥18-year-old with acute cough and fever	642 46.7-year-old	Prospective cohort study CRP method not informed CRP-testing within decision tree	Antibiotic prescription without pneumonia 48.3%. CRP-testing & decision tree 39.2% (p <.001). Pneumonia: CRP <10: 0%; CRP 11-50: 20%; CRP 50-100: 22%; CRP >100: 57%
Cals et al., 2011 [288]	The Netherlands	20 general practices 2005-2007	≥18-year-old with LRTI	431 ~ 50-year-old	Individually randomised factorial trial CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway); pneumonia & CRP cut-offs 20; 20-50; 50-100 and >100 mg/L	Antibiotic prescription: CRP-testing 39.1% *vs* communication training 33.3% *vs* CRP & communication training 23.1% *vs* usual care 66.7%. Safety: re-consultations similar in all groups after 28 days
Jakobsen et al., 2010 [293]	Sweden, Norway and UK	23 primary care practices 2006-2007	>18-year-old with acute cough or LRTI	503 Age ≥65-year-old ~40%	Prospective observational study CRP-testing: NycoCard CRP Single Test, Axis-Shield PoC AS, Norway; QuickRead® CRP, Orion Diagnostica, Finland. CRP cut-offs <20, 20-50, ≥50 mg/L	65% CRP <20 Antibiotic prescription: Sweden & Norway with CRP-testing 35% *vs* no testing 36% *vs* Wales 70% (no testing). CRP <20: 19%, 20-49: 49%; ≥50: 88%
Neumark et al., 2010 [280]	Sweden	Primary healthcare centre 1999-2005	All patients with a diagnosis of RTI	146,000 27-year-old	Retrospective study based on patient's electronic record CRP-testing method not informed	Antibiotic prescription 45%: pneumonia 59%, sore throat 65%, influenza 8%, common cold 16%. Pneumonia & CRP ≥50: antibiotic ~80% *vs* CRP <50: ~65%; Acute bronchiolitis & CRP ≥50: antibiotic 92% *vs* CRP <50: antibiotic 54%.
Cals et al., 2010 [115]	The Netherlands	11 family practice centres 2007-2008	≥18-year-old with LRTI or rhinosinusitis	258 ~44-year-old	Individually randomised controlled trial CRP-testing: Orion Diagnostica, Finland. CRP cut-offs <20 (no antibiotic recommended), 20-99, (delayed antibiotic recommended), ≥50 mg/L (recommended)	CRP median; 54.3% with CRP <20; 7.4% with CRP >100. Antibiotic prescription: CRP-testing 43.4% *vs* control group 56.6%, RR 0.77 (0.56-0.98). Safety: similar in both groups on day 7 and after 28 days
Cals et al., 2009 [114]	The Netherlands	20 general practices 2005-2007	>18-year-old with suspected LRTI with cough <4 weeks	431 ~ 50-year-old	Pragmatic cluster randomised factorial controlled trial CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway). CRP cut-offs 20 and 100 mg/L	69% with CRP <20; 7% >100. Antibiotic prescription: CRP-testing 31% *vs* control group 53% (p =0.02); 27% with communication training *vs* 54% without (p<.001). Combination CRP & communication 23%. CRP-testing *vs* communication non-different (p =0.78). Safety similar between both groups.

Takemura et al., 2005 [295]	Japan	General/Internal medicine clinic of a hospital 2000-2003	T ≥37.5˚C with suspicion of an infection <8 days	305 ~35-year-old	Prospective randomised controlled trial CRP-testing: model TBA-30FR; Toshiba, Japan	Antibiotic prescription: CRP & WBC-testing 51.7% *vs* no testing 87.7%; non-pneumonic acute RTI: CRP-testing 58% *vs* no CRP-testing 91% Influenza: CRP-testing 19% *vs* no CRP-testing 36%; acute gastroenteritis: CRP-testing 20% *vs* no CRP-testing 18%; total: CRP-testing 47% *vs* no CRP-testing 79%. Safety: no significant difference
Engström et al., 2004 [281]	Sweden	12 primary healthcare centres 2001	All patients with RTI	19,965 33-year-old	Retrospective study of electronic patient records CRP-testing: Orion Quickread CRP (Orion Diagnostica, Finland)	Antibiotic prescription: CRP-testing 37% *vs* no testing 41% (p <.001); 21% if CRP <25; 61% of CRP 25-50; 83% if CRP 50-100; 80% if CRP >100. Antibiotic prescription with CRP tests: 16-43%. 68% of CRP tests finally labelled as URTI
André et al., 2004 [302]	Sweden	155 primary healthcare centres 2000-2002	All patients with RTI	6,778 NA	Prospective study CRP-testing: NycoCard CRP Single Test, Axis-Shield PoC AS, Norway; QuickRead® CRP, Orion Diagnostica, Finland.	50% with CRP <10; 17% with CRP ≥50 and 7% with CRP ≥100. Decreased CRP for increased length of symptoms (p <.001). Antibiotic prescription: CRP-testing 41% *vs* no CRP-testing 44% (p <.001). Common cold: CRP <10, antibiotic prescription 4% *vs* CRP >50, antibiotic prescription 68%; Viral RTI: no difference antibiotic prescription between CRP-testing and no testing; CRP <25, antibiotic prescription 12% *vs* CRP ≥25, antibiotic prescription 59%. Viral LRTI: antibiotic prescription higher in CRP-testing than no testing (p <.001).
Bjerrum et al., 2004 [297]	Denmark	367 general practitioners 2001-2002	Patients with suspected sinusitis	1,444 ~40-year-old	Prospective observational study CRP-testing: methods not informed	~50% with CRP <10. Antibiotic prescription: CRP-testing 59% *vs* testing 78%, OR 0.43 (0.33-0.58), and 80% when CRP >25.
Takemura et al., 2004 [294]	Japan	General/Internal medicine clinic of a hospital 2000-2003	T ≥37.5˚C with suspicion of an infection <8 days	305 ~35-year-old	Randomised controlled trial CRP-testing: model TBA-30FR; Toshiba, Japan	CRP median 19. Antibiotic prescription: CRP & WBC-testing 61% *vs* no testing 92% (p <.001). Antibiotic prescription: 83% if CRP ≥40 *vs* 37% if CRP <40. Safety: no differences in early revisits
Diederichsen et al., 2000 [287]	Denmark	35 general practices centres 1997	Respiratory infection without chronic inflammatory diseases	812 37-year-old	Individually randomised controlled trial CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway). CRP cut-offs 10 and 50 mg/L	62% patients with chest abnormalities, 28% of patients with CRP ≤10. Antibiotic prescription: CRP-testing 43% *vs* control group 46%, OR 0.9 (0.7-1.2); 25% prescription if CRP ≤10. CRP impact on antibiotic prescription: OR 1.1 per unit increase (mg/L), p <.001.
Dahler-Eriksen et al., 1999 [304]	Denmark	29 clinics 1996	All primary healthcare patients	1,853 53.7-year-old	Open randomised crossover trial Controls: CRP available by mail Intervention: CRP available at POC CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway)	Higher CRP use for "new diseases" and "infectious diseases"; Antibiotic prescription: no difference between controls and intervention. Increased prescription with increased CRP levels. Patients' delay for collecting antibiotics at pharmacy: CRP-testing 2.3% *vs* no testing 16.7% (p =0.0161).

Melbye et al., 1995 [116]	Norway	10 primary care practices NA	>18-year-old with suspicion LRTI without sore throat, blocked nose or pain in ears/sinuses	239 NA	Individually randomised controlled trial CRP-testing: quantitative reader Nycocard Reader II (Axis Shield, Norway). CRP cut-offs 10, 50 and >50 mg/L for different durations of illness	Antibiotic prescription: CRP-testing 56% *vs* control 60% (p ≥.05). Safety: no difference on day 7 (RR 0.94 [0.75-1.18]) nor day 21 (RR 0.85 [0.57-1.29])
Keitel et al., 2019						Comment to a study [100]
Verbakel et al., 2019						Review of the literature
Bowe et al., 2019						Evaluation on hospital stay
Bryndonckx et al., 2018						No CRP evaluation on antibiotic prescription
Haenssgen et al., 2018						Qualitative analysis
Wang et al., 2018						No CRP-guiding treatment
Musson et al., 2018						Comment to a study [455]
van den Berg et al., 2018						Comment to a study [455]
Cals et al., 2018						Comment to a study [284]
Bell et al., 2017						Methods for optimising CRP impact
Tonkin-Crine et al.,2017						Overview of systematic reviews
Ebell et al.,2017						CRP evaluation not on antibiotic prescription
Haldrup et al.,2017						CRP evaluation not on antibiotic prescription
Hardy et al.,2017						Qualitative analysis
Tokodai et al.,2017						CRP evaluation not on antibiotic prescription
Brink et al.,2016						CRP review in antibiotic stewardship
Wagner et al.,2016						Update on outpatient's management
Meili et al.,2016						CRP evaluation not on antibiotic prescription
Moberg et al.,2016						CRP evaluation not on antibiotic prescription
Kimura et al.,2016						CRP evaluation not on antibiotic prescription
Lindström et al., 2015						Qualitative analysis
Meili et al., 2015						Review of the literature
Hunter et al.,2015						Cost-effectiveness analysis
Aabenhus et al.,2014						Review of the literature
Lemiengre et al., 2014						Design & Methods description
Tonkin-Crine et al., 2014						Qualitative analysis

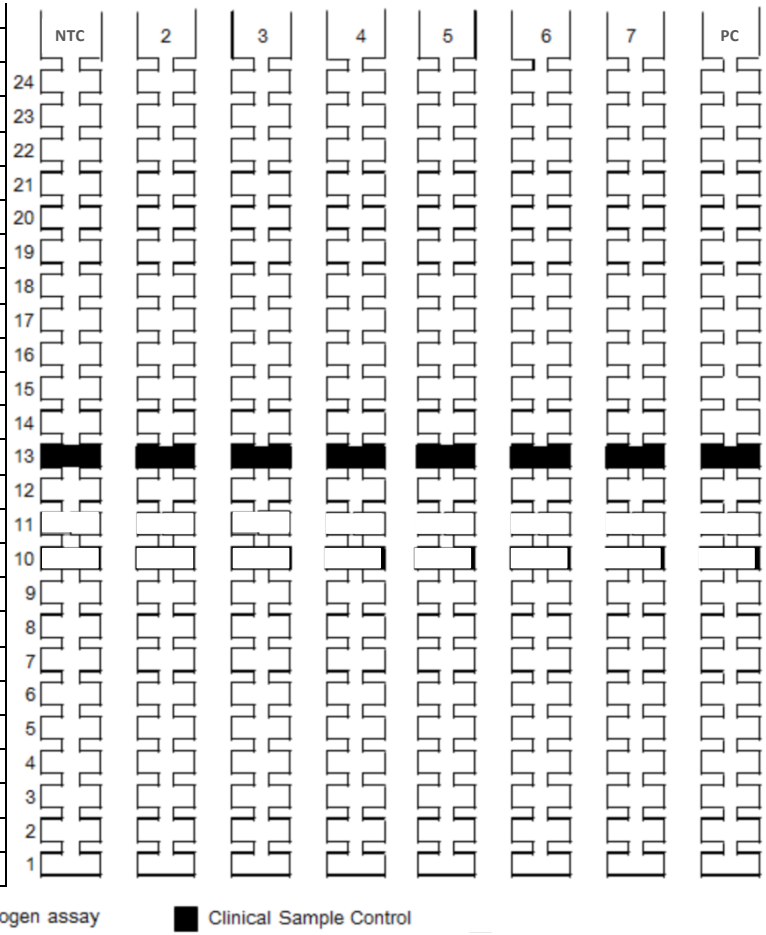
Chirillo et al., 2013	Protocol description
Oppong et al., 2013	Cost-effectiveness analysis
Joshi et al., 2013	Systematic review
Huang et al., 2013	Systematic review
Sims et al., 2013	Under 50 participants
Llor et al., 2013	Only abstract in English
Allen et al.,2013	Case report
Coenen et al., 2012	No CRP evaluation
Engel et al., 2012	Review of the literature
Llor et al., 2011	No CRP evaluation
Kimura et al., 2011	Case report
Llor et al., 2010	No CRP evaluation on antibiotic prescription
Lillie et al., 2010	CRP evaluation about treatment duration
Elsurer et al., 2010	Case report & literature review
Sakao et al., 2008	Case report
Saranchuk et al., 2008	No evaluation of CRP impact on antibiotic prescription
Schaaf et al., 2007	CRP evaluation for severity
Hoare et al., 2006	Review of the literature
Takemura et al., 2005	Cost-effectiveness analysis
Mölstad et al., 2003	No CRP evaluation
Stockley et al., 2000	No CRP evaluation
Yorioka et al., 1998	Under 50 participants

AECOPD: acute exacerbation of chronic obstructive pulmonary disease; ALMANACH: algorithm for the management of acute childhood illnesses; AOM: acute otitis media; ARI: acute respiratory infection; BISNA: brief intervention to elicit parental concern combined with safety net advice; COPD: chronic obstructive pulmonary disease; CURB-65: confusion, uraemia ≥20 mg/dL, respiratory rate ≥30 breaths/min, systolic blood pressure >90 mmHg and age ≥65-year-old; CRP: C-reactive protein; e-POCT: electronic point-of-care test; GRACE network includes: UK, Norway, Sweden, Finland, Belgium, the Netherlands, Germany, Spain, Italia, Portugal, Switzerland, Slovakia and Hungary; HR: hazard ratio; IMCI: integrated management of childhood illness; LRTI: lower respiratory tract infection; OHPAT: outpatient and home parenteral antibiotic therapy; OPD: outpatient department; OR (95% CI): odds ratio with 95% confidence interval; POC: point-of-care; RTI: respiratory tract infection; RR: relative risk; POCT: point-of-care test; PPPY: physician per patient per year; T: temperature; URTI: upper respiratory tract infection; WBC: white blood cell count

APPENDIX VI

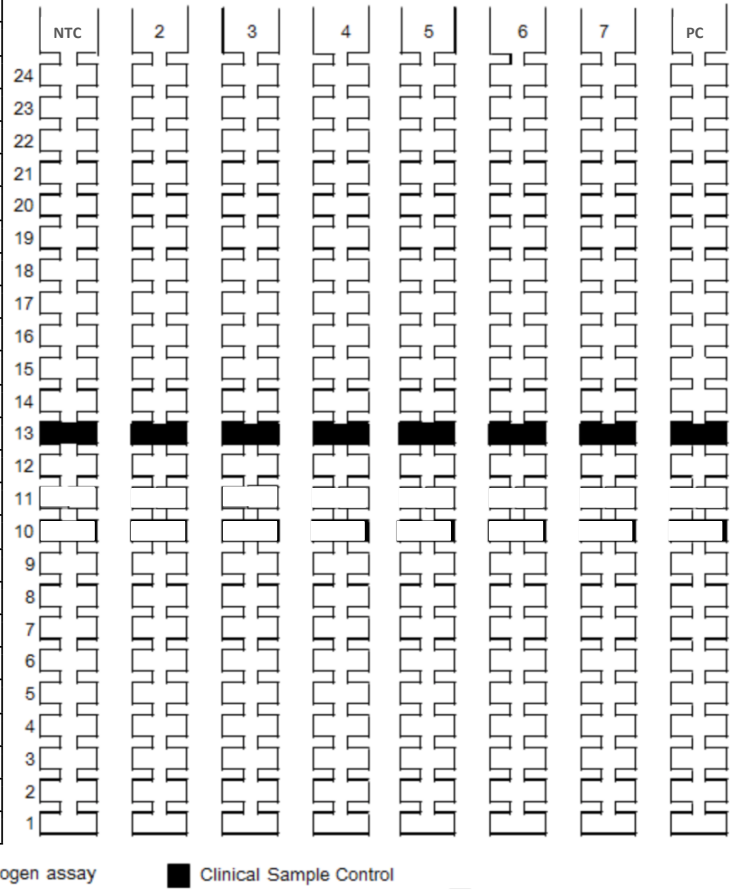
Appendix VI. A Configuration of the TaqMan Array Card for detection of the organisms in blood specimens

Port	
Left	**Right**
Dengue / Chikungunya / Zika	Dengue / Chikungunya / Zika
Dengue / Chikungunya / Zika	Dengue / Chikungunya / Zika
Salmonella spp. / *Escherichia coli* / *Shigella* spp.	*Salmonella* spp. / *Escherichia coli* / *Shigella* spp.
Salmonella spp. / *Escherichia coli* / *Shigella* spp.	*Salmonella* spp. / *Escherichia coli* / *Shigella* spp.
Burkholderia pseudomallei	*Burkholderia pseudomallei*
Burkholderia pseudomallei	*Burkholderia pseudomallei*
Group A *Streptococcus* / *Staphylococcus aureus*	Group A *Streptococcus* / *Staphylococcus aureus*
Group A *Streptococcus* / *Staphylococcus aureus*	Group A *Streptococcus* / *Staphylococcus aureus*
Klebsiella pneumoniae / *Haemophilus influenzae*	*Klebsiella pneumoniae* / *Haemophilus influenzae*
Klebsiella pneumoniae / *Haemophilus influenzae*	*Klebsiella pneumoniae* / *Haemophilus influenzae*
Plasmodium falciparum / *Plasmodium vivax*	*Plasmodium falciparum* / *Plasmodium vivax*
Internal Positive control (IPCO)	Human control (RNP3)
Group B *Streptococcus* / *H. influenzae* type B	Group B *Streptococcus* / *H. influenzae* type B
Varicella zoster virus/Measles virus	Varicella zoster virus/Measles virus
Bartonella spp. / *Brucella* spp. / *Yersinia* spp.	*Bartonella* spp. / *Brucella* spp. / *Yersinia* spp.
Enterovirus / Adenovirus	Enterovirus / Adenovirus
Nipah virus / *Streptococcus suis*	Nipah virus / *Streptococcus suis*
Japanese encephalitis virus	Japanese encephalitis virus
Rhinovirus	Rhinovirus
Bocavirus	Bocavirus
Salmonella enterica Paratyphi A/*O.tsutsugamushi*	*Salmonella* enterica Paratyphi A/*O.tsutsugamushi*
Salmonella enterica Typhi/*Listeria monocytogenes*	*Salmonella* enterica Typhi/*Listeria monocytogenes*
Leptospira (pan serovar) / Hepatitis E	*Leptospira* (pan serovar) / Hepatitis E
Parechovirus / Rubella virus / *Rickettsia* spp.	Parechovirus / Rubella virus / *Rickettsia* spp.



Appendix VI. B Configuration of the TaqMan Array Card for detection of the organisms in nasopharyngeal swabs.

Port	
Left	**Right**
Pseudomonas aeruginosa / Streptococcus pneumoniae	*Pseudomonas aeruginosa / Streptococcus pneumoniae*
Chlamydia trachomatis / Burkholderia pseudomallei	*Chlamydia trachomatis / Burkholderia pseudomallei*
Varicella zoster virus / Measles virus	Varicella zoster virus / Measles virus
Parainfluenza 1 / Parainfluenza 3	Parainfluenza 1 / Parainfluenza 3
MERS Coronavirus	MERS Coronavirus
Coronavirus NL63 / Coronavirus HKU1	Coronavirus NL63 / Coronavirus HKU1
Coronavirus 229E / Coronavirus OC43	Coronavirus 229E / Coronavirus OC43
Influenza A / Influenza B	Influenza A / Influenza B
Enterovirus / Adenovirus	Enterovirus / Adenovirus
Klebsiella pneumoniae / Haemophilus influenzae	*Klebsiella pneumoniae / Haemophilus influenzae*
Group A Streptococcus / Staphylococcus aureus	*Group A Streptococcus / Staphylococcus aureus*
Internal positive control (IPCO)	Human control (RNP3)
Chlamydophila pneumoniae / Mycoplasma pneumoniae	*Chlamydophila pneumoniae / Mycoplasma pneumoniae*
Rhinovirus	Rhinovirus
Human Metapneumovirus	Human Metapneumovirus
Corynebacterium diphtheriae triplex	*Corynebacterium diphtheriae* triplex
Bordetella pertussis / Bordetella parapertussis	*Bordetella pertussis / Bordetella parapertussis*
Respiratory syncytial virus / Cytomegalovirus	Respiratory syncytial virus / Cytomegalovirus
Bocavirus	Bocavirus
Moraxella catarrhalis / Acinetobacter baumannii	*Moraxella catarrhalis / Acinetobacter baumannii*
Hepatitis E virus / Rubella virus duplex	Hepatitis E virus / Rubella virus duplex
Pneumocystis jirovecii	*Pneumocystis jirovecii*
Leptospira (pan serovar)	*Leptospira* (pan serovar)
Escherichia coli / Shigella spp.	*Escherichia coli / Shigella* spp.



APPENDIX VII

Appendix VII Taqman array card (TAC): within respiratory card reproducibility

Assay	Ct*	% CV**
Acinetobacter baumannii	38.57	8.2
Corynebacterium diphtheriae	32.00	0.19
Chlamydophila pneumoniae	28.88	1.56
Group A *Streptococcus*	32.30	1.40
Haemophilus influenzae	29.50	0.75
Klebsiella pneumoniae	34.55	1.99
Moraxella catarrhalis	28.96	0.78
Mycoplasma pneumoniae	30.48	0.95
Pseudomonas aeruginosa	35.53	1.39
Staphylococcus aureus	32.68	1.10
Streptococcus pneumoniae	30.67	3.18
Adenovirus	36.55	1.86
Bocavirus	31.97	2.05
Rhinovirus	29.31	0.95
Enterovirus	34.30	1.48
Human coronavirus	30.11	0.86
Influenza type A	23.79	0.71
Influenza type B	24.61	0.44
Parainfluenza (1&3)	28.11	0.81
Human metapneumovirus	28.34	0.65
Respiratory syncytial virus	28.66	0.55
Cytomegalovirus	34.62	3.75
Varicella-Zoster virus	33.36	1.06

*Ct indicates the mean of the two cycle thresholds, as each organism was tested twice
**CV indicates the coefficient of variation

APPENDIX VIII

Appendix VIII Evidence review for classifying influenza virus A & B, respiratory syncytial virus, human metapneumovirus and *Bordetella pertussis* as pathogenic in nasopharyngeal swabs.

Organism	Methods	Findings	References
Influenza virus A & B	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 3.6, 95% CI (2.4-5.3)	[368]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	Influenza A: OR 1.5, 95% CI (0.8-2.7) Influenza B: OR 3.8, 95% CI (1.2-12.4)	[62]
	Multi-site case-control study among children and adults with SARI or ILI	AF 86.3%, 95% CI (77.7-91.6%)	[373]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 4.13, 95% CI (2.06-8.26)	[460]
Respiratory syncytial virus (RSV)	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 14.0, 95% CI (11.4-17.1)	[368]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	OR 6.3, 95% CI (4.2-9.4)	[62]
	Multi-site case-control study among children and adults with SARI or ILI	AF 83.7%, 95% CI (77.5-88.2%)	[373]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 8.05, 95% CI (4.21-15.38)	[460]
Human metapneumovirus (hMPV)	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 6.3, 95% CI (4.8-8.2)	[368]
	Multi-site international case-control study among 0-59-day-old babies with community-acquired serious infections	OR 1.3, 95% CI (0.5-3.2)	[62]
	Multi-site case-control study among children and adults with SARI or ILI	AF 85.6%, 95% CI (72.0-92.6%)	[373]
	Nested case-control study among children 0-42-month-old with pneumonia	OR 1.12 (0.67-1.88)	[460]
Bordetella pertussis	Multi-site international case-control study among 1-59-month-old children with respiratory symptoms	OR 3.3, 95% CI (1.6-7.2)	[368]
	Nested case-control study among 0-42-month-old children with pneumonia	OR 11.08, 95% CI (1.33-92.54)	[460]

ILI – Influenza-like illness
SARI – Severe acute respiratory infection
OR – Odds ratio
AF – Attributable fraction

APPENDIX IX

Effect of point-of-care C-reactive protein testing on antibiotic prescription in febrile patients attending primary care in Thailand and Myanmar: an open-label, randomised, controlled trial

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Summary

Background In southeast Asia, antibiotic prescription in febrile patients attending primary care is common, and a probable contributor to the high burden of antimicrobial resistance. The objective of this trial was to explore whether C-reactive protein (CRP) testing at point of care could rationalise antibiotic prescription in primary care, comparing two proposed thresholds to classify CRP concentrations as low or high to guide antibiotic treatment.

Methods We did a multicentre, open-label, randomised, controlled trial in participants aged at least 1 year with a documented fever or a chief complaint of fever (regardless of previous antibiotic intake and comorbidities other than malignancies) recruited from six public primary care units in Thailand and three primary care clinics and one outpatient department in Myanmar. Individuals were randomly assigned using a computer-based randomisation system at a ratio of 1:1:1 to either the control group or one of two CRP testing groups, which used thresholds of 20 mg/L (group A) or 40 mg/L CRP (group B) to guide antibiotic prescription. Health-care providers were masked to allocation between the two intervention groups but not to the control group. The primary outcome was the prescription of any antibiotic from day 0 to day 5 and the proportion of patients who were prescribed an antibiotic when CRP concentrations were above and below the 20 mg/L or 40 mg/L thresholds. The primary outcome was analysed in the intention-to-treat and per-protocol populations. The trial is registered with ClinicalTrials.gov, number NCT02758821, and is now completed.

Findings Between June 8, 2016, and Aug 25, 2017, we recruited 2410 patients, of whom 803 patients were randomly assigned to CRP group A, 800 to CRP group B, and 807 to the control group. 598 patients in CRP group A, 593 in CRP group B, and 767 in the control group had follow-up data for both day 5 and day 14 and had been prescribed antibiotics (or not) in accordance with test results (per-protocol population). During the trial, 318 (39%) of 807 patients in the control group were prescribed an antibiotic by day 5, compared with 290 (36%) of 803 patients in CRP group A and 275 (34%) of 800 in CRP group B. The adjusted odds ratio (aOR) of 0·80 (95% CI 0·65–0·98) and risk difference of –5·0 percentage points (95% CI –9·7 to –0·3) between group B and the control group were significant, although lower than anticipated, whereas the reduction in prescribing in group A compared with the control group was not significant (aOR 0·86 [0·70–1·06]; risk difference –3·3 percentage points [–8·0 to 1·4]). Patients with high CRP concentrations in both intervention groups were more likely to be prescribed an antibiotic than in the control group (CRP ≥20 mg/L: group A *vs* control group, p<0·0001; CRP ≥40 mg/L: group B *vs* control group, p<0·0001), and those with low CRP concentrations were more likely to have an antibiotic withheld (CRP <20 mg/L: group A *vs* control group, p<0·0001; CRP <40 mg/L: group B *vs* control group, p<0·0001). 24 serious adverse events were recorded, consisting of 23 hospital admissions and one death, which occurred in CRP group A. Only one serious adverse event was thought to be possibly related to the study (a hospital admission in CRP group A).

Interpretation In febrile patients attending primary care, testing for CRP at point of care with a threshold of 40 mg/L resulted in a modest but significant reduction in antibiotic prescribing, with patients with high CRP being more likely to be prescribed an antibiotic, and no evidence of a difference in clinical outcomes. This study extends the evidence base from lower-income settings supporting the use of CRP tests to rationalise antibiotic use in primary care patients with an acute febrile illness. A key limitation of this study is the individual rather than cluster randomised study design which might have resulted in contamination between the study groups, reducing the effect size of the intervention.

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Introduction

Southeast Asia is a global hub of antimicrobial resistance, which is associated with high morbidity and mortality,[1] and is a probable exporter of antimicrobial resistance through dense travel and agricultural trade networks.[2] With an extensive population reach, primary care could be a major force in the fight against antimicrobial resistance; instead, primary care is a setting in which antibiotic prescription is widespread and poorly regulated.[3] Challenges include high patient throughput, diagnostic uncertainty, patient expectations of treatment, and reluctance to refrain from prescribing when follow-up is challenging.[4–6] A situational analysis of antimicrobial resistance in southeast Asia reported that in Myanmar 87% of patients with upper respiratory tract infections in primary care received an antibiotic, as did 43% of patients in Thailand. A review of 32 primary care centres in northern Thailand found that 47·6% of patients with a documented fever were prescribed antibiotics.[7,8]

Fever is a common reason for attending primary care facilities. Although malaria can be readily ruled out with rapid tests, the ubiquitous use of these tests as malaria declines implies that most febrile patients will have a negative test result. Health-care providers in primary care, however, have no means to diagnose other causes of acute fever, driving further inappropriate antibiotic prescription.[9] Concurrently, patients with potentially life-threatening bacterial diseases such as scrub typhus and leptospirosis, which are widespread in southeast Asia, often receive no treatment or inappropriate antibiotics.[10,11] Ideally, pathogen-specific rapid tests would establish whether and which antibiotics are required at point of care, but use of these tests is unlikely to be feasible in the foreseeable future because of the small range of point-of-care tests that are available, with many tests being unable to distinguish between invasive infection and past exposure. Furthermore, even well resourced research studies using laboratory reference tests and paired samples rarely identify a pathogenic agent in more than half of febrile patients.[10–12]

Point-of-care tests for host-response biomarkers offer an alternative to pathogen-specific testing, with the potential for ruling out the need for antibiotic treatment and reassuring health-care providers and patients when this treatment is less likely to be required. The need for simple tests to assist in prescribing decisions has been recognised globally,[13] but few potential biomarkers have been evaluated across a broad range of settings and

Research in context

Evidence before this study

Retrospective studies have found C-reactive protein (CRP) to be highly sensitive and moderately specific in the identification of bacterial infection in blood samples from febrile patients. One such study of over 1300 microbiologically confirmed infections in patients across southeast Asia found a sensitivity of 86% and a specificity of 67% for CRP at a threshold of 20 mg/L, with an area under the curve of the receiver operating characteristic of 0·83 (95% CI 0·81–0·86). A Cochrane systematic review of six studies including more than 3000 patients in primary care who presented with acute respiratory infections in high-income settings concluded that CRP was an effective measure to reduce antibiotic prescription. Another systematic review of clinical trials of host biomarker testing for the identification of serious infections in children concluded that CRP tests could be diagnostically useful, but more evidence was needed on specific thresholds. A cluster randomised controlled trial from Belgium concluded that CRP testing should be targeted at children at risk of severe infections. Neither this study nor those in the systematic reviews originate in low-income and middle-income countries (LMICs) where the burden of infectious diseases is high and access to well trained clinicians can be low. We searched MEDLINE for studies published in English using the combination of "trial", "fever" or "febrile", and "C reactive protein" and identified two relevant trials from LMICs. We applied no date restriction to our search and our last search was Jan 20, 2018. In Tanzania, CRP testing was incorporated within a bundle of interventions that resulted in a large reduction in antibiotic prescribing in children attending outpatient clinics from a baseline of 94·9% to just 11·5%. In Vietnam, CRP testing alone in patients with acute respiratory tract infections reduced antibiotic prescribing from 78% in the control group to 64% in the intervention group.

Added value of this study

We extended the evaluation of CRP testing in southeast Asian primary care settings to all acutely febrile patients older than 12 months. The study included two intervention groups with different CRP thresholds indicating the need for antibiotics. The findings suggest that only the higher threshold of 40 mg/L was associated with significant reductions in prescribing compared with the control group, although all three study groups had significantly lower antibiotic prescription than those documented in retrospective surveys before the trial. In both intervention groups, patients with elevated CRP were more likely to be prescribed an antibiotic than those in the control group, providing an additional diagnostic safety layer that could be particularly important in settings where access to well trained clinicians is low.

Implications of all the available evidence

In primary care settings in southeast Asia where the prevalence of antibiotic prescription is high, CRP testing can be used to inform the management of patients with an acute fever and those with an acute respiratory tract infection.

	Chiangrai, Thailand	Hlaing Tha Yar, Myanmar
Sites	Six public primary care units	Three primary care clinics and one outpatient department (government hospital)
Location	Rural and peri-urban settings within a 30 km radius of Chiangrai city centre	Slum areas and peri-urban townships on the west side of Yangon
Health-care provider	Two to three registered nurses per site	Two to five medical doctors per site
Access fees	Universal health coverage for registered citizens*	Free
Population	Thai community, 15% ethnic minorities	Mainly Burmese community
Investigations routinely available	Finger-prick blood glucose test	Rapid test for malaria
Malaria transmission	0–0·1 cases per 1000 population	0–0·1 cases per 1000 population

*THB30 (US$0·91) were previously charged per visit; this fee is now inconsistently applied.

Table 1: **Trial sites**

populations, and none has shown perfect diagnostic performance.[14,15]

C-reactive protein (CRP) is an acute-phase biomarker of inflammation. Although increases in CRP concentrations have a variety of causes,[16] the utility of CRP in distinguishing between bacterial and viral infections has been shown in stored samples from febrile patients in southeast Asia across diverse settings and populations, including inpatients, outpatients, children, adults, and pregnant women.[17,18] These studies concluded that CRP is highly sensitive and moderately specific in identifying bacterial infections. Although research and development for better biomarkers continues, CRP testing is potentially a readily available means of improving prescribing decisions as a plethora of CRP point-of-care tests are commercially available,[19] with some costing less than US$1·00.[20] However, selecting a CRP test for use in routine care requires the identification of optimal thresholds to indicate the need for antibiotic treatment, for which scant evidence is available.

A clinical trial in Vietnamese patients with acute respiratory tract infections in primary care[21] found that CRP testing with a threshold of 10 mg/L in children and 20 mg/L in adults reduced antibiotic prescription from 78% to 64% without altering the duration of symptoms. The objective of our clinical trial was to estimate the effect of CRP testing on antibiotic prescription in acutely febrile children and adults attending primary care in Thailand and Myanmar. Previous trials of CRP-guided antibiotic treatment used quantitative readers, which are unlikely to be available in many low-income and middle-income settings. In our trial, health-care providers were notified only as to whether CRP concentrations were low or high with respect to two proposed thresholds—20 mg/L and 40 mg/L. Identifying an optimal threshold for CRP-guided antibiotic treatment could inform the choice of lateral flow devices for use in routine primary care settings.

Methods

Study design and participants

This study was designed as a multicentre, open-label, randomised, controlled trial that compared CRP-guided antibiotic prescription in febrile patients with the standard prescribing practice. The design included two intervention groups with CRP thresholds of 20 mg/L and 40 mg/L to guide antibiotic prescription. These thresholds were selected on the basis of previous literature on CRP concentrations in febrile patients,[22,23] particularly studies from southeast Asia,[17,18] including a study from Chiangrai, Thailand, that evaluated 20 mg/L and 40 mg/L CRP as candidate thresholds for the identification of bacterial infections (sensitivity of 92% for 20 mg/L and 86% for 40 mg/L).[24]

In Thailand, primary care units (PCUs) in Chiangrai were selected as study sites for patient recruitment, with the intention of including facilities with high patient turnover while ensuring diversity in terms of the rural or urban environment. Two PCUs were initially included, and four additional sites were later opened because of slow recruitment. In Myanmar, the study was done in three Medical Action Myanmar (MAM) clinics and in one adjacent hospital outpatient department located in the poorest township of Yangon (table 1).[25]

In terms of policy environment and antimicrobial resistance awareness campaigns, Thailand has been notably active compared with other countries in the region,[7] including through its Antibiotic Smart Use (ASU) programme, in place since 2007.[26] The programme set a target prescription rate of 20% per month for respiratory infections and acute diarrhoea as part of the key performance indicators for PCUs; this target has been integrated in a pay-for-performance (P4P) policy of the National Health Security Office since 2009. In August, 2016 (during the study), the National Strategic Plan on antimicrobial resistance 2017–21 was endorsed by the cabinet as Thailand's first national strategy addressing antimicrobial resistance challenges, and the Ministry of Public Health (MOPH) adopted the ASU targets into its Service Plan Policy on Rational Drug Use (RDU).[27] Unlike the previous P4P policy that provided financial incentives at the facility level, the RDU Service Plan incentivised higher-level stakeholders in the MOPH to exercise their authority in meeting the antibiotic prescription targets. To achieve this, the MOPH now relies on health

inspectors to guide and encourage hospitals and PCUs to reduce the unnecessary use of antibiotics. The antimicrobial-resistance policy environment in Myanmar is not well developed, with no relevant policies introduced between 2010 and 2015.[7]

In addition to the influence of the shifting policy environment, we anticipated a potential for observation bias due to the presence of research staff and a possible contamination effect on prescribing in the control group resulting from exposure to CRP test results in the intervention groups (ie, if healthcare providers observe low frequency of patients with high CRP in the interventions groups, this might affect their prescribing in patients in the control group). Therefore, to understand the prescribing practices in febrile patients in the trial sites before intervention, we did surveys to include retrospective data from January, 2015, to December, 2016, in Thailand, and from November, 2015, to April, 2016, in Myanmar. Further details on the data collection processes for the background surveys are presented in the appendix.

See Online for appendix

All participants in the trial were aged 1 year or older with a documented fever (defined as a tympanic temperature of >37·5°C according to WHO standards) or a chief complaint of fever (<14 days), regardless of previous antibiotic intake and comorbidities other than malignancies. The exclusion criteria were as follows: infants younger than 1 year; symptoms requiring hospital referral, defined as either impaired consciousness, inability to take oral medication, or convulsions; a positive malaria test; the main complaint being trauma or injury; suspicion of either tuberculosis, urinary tract infection, or local skin or dental abscess or infection; any symptom present for more than 14 days; any bleeding; and an inability to comply with the follow-up visit at day 5. A complete list of all inclusion and exclusion criteria can be found in the protocol summary on ClinicalTrials.gov. All participants (or parents or guardians in the case of children) provided written informed consent. The protocol, informed consent form, and case record forms were reviewed and approved by the Oxford Tropical Research Ethics Committee, the Mahidol University Faculty of Tropical Medicine Ethics Committee, and the Myanmar Department of Medical Research and Chiangrai Provincial Public Health Office Research Ethics Committees.

For a **summary of the protocol** see https://clinicaltrials.gov/ct2/show/NCT02758821

Study staff explained the trial to potential participants and took their written informed consent to join the study before any study-specific procedures were done. In the case of participants younger than 18 years, a parent or guardian was asked to sign and date the informed consent form (ICF) and the participant was asked to give consent or assent depending on their age and local practice. In the case of illiterate patients or parents or guardians, a witness was asked to sign the ICF to confirm that the participant gave informed verbal consent to participate.

For the **educational video on antimicrobial resistance and CRP** see https://www.youtube.com/watch?v=hJnxSwbqWOE&feature=youtu.be

Randomisation and masking

Individuals were randomly assigned at a 1:1:1 ratio to either one of the two intervention groups or the control group and were stratified by country (Thailand and Myanmar) and age group (children and adults, with adulthood defined as age ≥12 years). Computer-based individual randomisation was done at the Mahidol-Oxford Tropical Medicine Research Unit, Bangkok, Thailand by the trial statistician (MM) using the ralloc command in Stata version 14. Numbered, sealed, opaque envelopes containing the randomised study group were prepared by the central support team and opened sequentially on site by the study staff after patients were enrolled. Study groups consisted of intervention groups A and B, in which CRP was measured by study staff on site and the result was communicated to the health-care provider as low CRP or high CRP using cutoff thresholds of 20 mg/L (group A) or 40 mg/L (group B), and control group C, in which health-care providers were asked to manage febrile patients as per standard of care.

Health-care providers and patients were masked to allocation between the two intervention groups but by design were aware of allocation to the control group. Research staff could not be masked to patient allocation between all groups.

Procedures

Before patient recruitment, the health-care providers were informed of the utility of CRP to help guide antibiotic prescription, while mitigating the threat of antimicrobial resistance. The information was provided by the study investigators in the local language and dialect, on the basis of previous models developed for CRP-testing-related training,[28] and contextualised to the Thai and Myanmar settings. A refresher session was provided around the midpoint of study recruitment. Health-care providers were advised that for febrile patients with no clear danger signs and low CRP concentrations they should refrain from prescribing antibiotics, whereas for patients with high CRP the guidance was to consider prescribing antibiotics on the basis of their clinical judgment. The health-care providers were informed that the test was not of perfect accuracy in its identification of patients requiring antibiotic treatment.

Following randomisation, patients from the intervention groups had a capillary blood sample analysed for CRP on site by the study staff who used a CRP reader (NycoCard II Reader, Axis Shield, Oslo, Norway). A brief educational video on antimicrobial resistance and CRP was shown to participants in the intervention groups with the intention of ensuring patients' understanding of the test. The participants were then provided with a card specifying whether their CRP concentrations were high or low in relation to their intervention group and referred to the health-care provider. In the control group, a venous blood sample was collected by study staff, stored at 4°C, and retrospectively tested for CRP concentrations. All patients then proceeded to a routine medical examination by the

primary-care health provider who decided whether an antibiotic was required. Demographic and clinical data were recorded by the study staff on a case report form (CRF).

All patients were followed up both at day 5 (allowable range 4–7 days) and day 14 (allowable range 12–16 days) after recruitment by face-to-face appointment with study staff. If patients were unable to attend a follow-up visit in person, a structured telephone interview was done instead. Patients in all three groups were tested for CRP at the second visit (day 5) on site by the study staff to help gauge clinical recovery, and health-care providers were informed if CRP concentrations were equal to or higher than 50 mg/L in children, and equal to or higher than 100 mg/L in adults.

Patients received compensation for their time and travel expenses at enrolment and on each of the follow-up visits if they reattended in person.

Outcomes

The primary outcome was the proportion of patients who were prescribed any antibiotic at the facility from day 0 to day 5 (allowable range 4–7) in each intervention group in total, and the proportion of patients who were prescribed an antibiotic when CRP concentrations were above and below the 20 mg/L or 40 mg/L thresholds. The CRP reading was done in a central lab off-site for the control group and it was performed on site for the intervention groups. The data on prescribing were recorded independently on site and the outcome was assessed centrally. Secondary outcomes included the proportion of patients prescribed an antibiotic from day 0 to day 14 at the health facility. Clinical outcomes included patient self-reported recovery at each follow-up visit, duration and severity of symptoms, frequency of unplanned reconsultation within the 14 days of follow-up, temperature and CRP concentrations at day 5 as objective measures of clinical recovery, and occurrence of serious adverse events, defined as events requiring admission to hospital or death within 14 days of enrolment. Due to the extensive trial outputs, this manuscript reports the primary outcome and key secondary outcomes relating to antibiotic prescribing and clinical recovery. The other

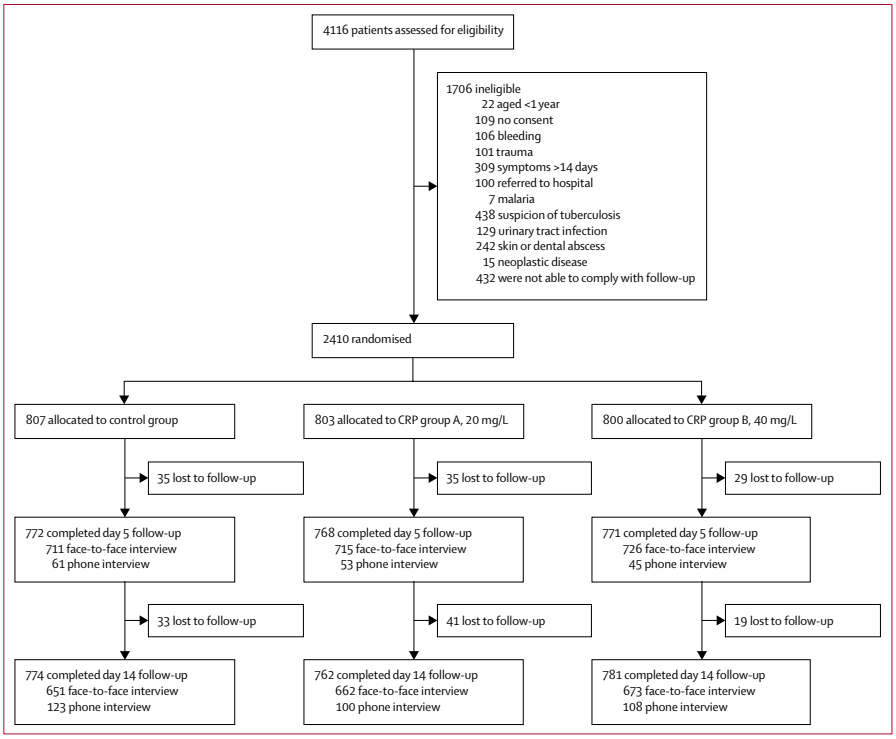

Figure 1: **Trial profile**
CRP=C-reactive protein.

	Control group		CRP group A		CRP group B	
	Aged <12 years (n=402)	Aged ≥12 years (n=405)	Aged <12 years (n=400)	Aged ≥12 years (n=403)	Aged <12 years (n=399)	Aged ≥12 years (n=401)
Demographic characteristics						
Sex						
Male	204 (51%)	159 (39%)	209 (52%)	156 (39%)	204 (51%)	174 (43%)
Female	198 (49%)	246 (61%)	191 (48%)	247 (61%)	195 (49%)	227 (57%)
Age, median (IQR), years	4 (2–7)	33 (22–52)	4 (2–7)	35 (20–53)	4 (2–7)	34 (21–51)
≥30 min to reach the facility	100 (25%)	66 (16%)	100 (25%)	69 (17%)	98 (25%)	81 (20%)
Presence of comorbidity*	15 (4%)	112 (28%)	16 (4%)	100 (25%)	20 (5%)	88 (22%)
Symptoms onset, median (IQR), days	2 (1–3)	3 (2–4)	2 (1–3)	3 (2–4)	2 (1–3)	3 (2–4)
Self-reported antibiotic intake	16 (4%)	25 (6%)	20 (5%)	17 (4%)	22 (6%)	29 (7%)
Clinical characteristics and self-reported symptoms						
Documented fever (>37·5°C)	200 (50%)	155 (38%)	203 (51%)	143 (35·5%)	223 (56%)	148 (37%)
Neurological symptoms†	62 (15%)	148 (37%)	39 (10%)	156 (39%)	40 (10%)	155 (39%)
Respiratory symptoms‡	326 (81%)	323 (80%)	315 (79%)	315 (78%)	327 (82%)	299 (75%)
Gastrointestinal tract symptoms§	104 (26%)	95 (23%)	124 (31%)	83 (21%)	109 (27%)	68 (17%)
Other symptoms¶	9 (2%)	25 (6%)	41 (10%)	37 (9%)	30 (8%)	43 (11%)

Data are number (%) or median (IQR). CRP=C-reactive protein. *Comorbidities included HIV infection, chronic hepatitis B or C infection, cirrhosis, diabetes, asthma, anaemia, chronic obstructive pulmonary disease, gastritis, congenital heart or kidney disease, alcoholism, dyslipidaemia, glucose-6-phosphate dehydrogenase deficiency, hypertension, rheumatic heart disease, thalassaemia, or thyroid disease. †Neurological symptoms include headache, confusion, dizziness, or hearing loss. ‡Respiratory symptoms include sore throat, dyspnoea, chest pain, runny nose, or cough. §Gastrointestinal symptoms include nausea, vomiting, diarrhoea, or abdominal pain. ¶Other symptoms declared were defined by the presence of fever alone or symptoms other than those present in neurological, respiratory, or gastrointestinal symptoms. Common symptoms in this group included myalgia, arthralgia, jaundice, tiredness, chills, sweating, weight loss, skin eruption, dysuria, or eye redness.

Table 2: **Day 0 characteristics comparing control group, group A (20 mg/L CRP threshold), and group B (40 mg/L CRP threshold)**

secondary outcomes of the study—the correlation between CRP results and clinical outcomes on day 5 of follow-up, the impact of CRP testing on antibiotic consumption obtained elsewhere after the first consultation, the attitudes and satisfaction of health-centre staff and patients towards the CRP test, the prevalence of key pathogens in febrile patients in these settings, and the ability of CRP to discriminate between viral and bacterial pathogens—will be reported elsewhere.

Statistical analysis

We expected CRP testing to reduce antibiotic prescriptions by 25% from baseline, but with anticipated contamination between study groups, the sample size was increased to detect a reduction in antibiotic prescription by 20% independently for children and adults in each country. The sample size was adjusted further to account for multiple comparisons between the three study groups on the basis of Bonferroni's correction. An adjusted significance level (type I error) of 0·017 was used to yield a 5% overall significance level for the three comparisons. Allowing for a projected 15% loss to follow-up required 198 patients per study group, rounded to 200, to give a total of 2400 patients (600 children and 600 adults per country).

The trial was analysed by intention to treat and per protocol (appendix). The per-protocol analysis included patients for whom follow-up data were available on both day 5 and day 14, and to whom health-care providers prescribed antibiotics in accordance with test results; therefore, the effect size in the per-protocol analysis represents the potential effect of the tests under full compliance.

Differences in primary and secondary outcomes were analysed overall and in the four predefined subgroups of country and age category. Descriptive statistics for continuous variables with normal distribution used means and SD and medians with IQR for non-normally distributed continuous variables. Comparison between groups used *t* tests for normally distributed variables, the Mann-Whitney test for non-normally distributed variables, and χ^2 test for categorical variables.

Primary and secondary outcomes between each of the intervention groups and the control group were compared using a logistic regression model; health facilities were considered to have a random effect on the primary outcome.

The difference in the number of prescriptions of various broad-spectrum antibiotics was also compared, including ceftriaxone, cefixime, ciprofloxacin, levofloxacin, azithromycin, and amoxicillin with clavulanic acid. The exhaustive list of antibiotics prescribed at the facilities is provided in the appendix. This analysis was not prespecified in the study protocol.

We generated Kaplan-Meier curves to visualise time to clinical recovery on the basis of the patient declaration, with a corresponding p value using a log-rank test for survival curves. We used a Cox regression model to quantify the difference in clinical outcomes

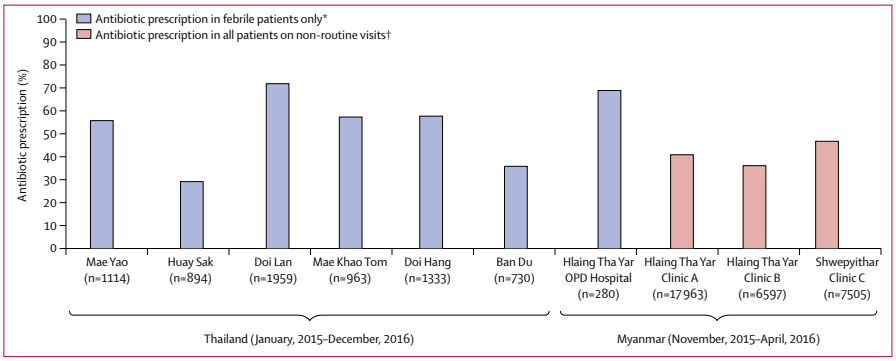

Figure 2: **Background antibiotic prescription in Thailand and Myanmar**
OPD=outpatient department. *Estimated prescriptions in patients for whom clinical data on febrile status were available. †Estimated prescriptions in all patients on non-routine visits (febrile status unknown).

between intervention and control groups calculating the adjusted hazard ratios (HRs), with age stratum and country as fixed effects and health-care centres as a Gaussian random effect. The 95% CIs have been provided where appropriate. Data analyses were done with Stata version 15. The Clinical Trial Support Group at the Mahidol Oxford Tropical Medicine Research Unit did two monitoring visits to each site to ensure the integrity of the data; the first visit took place after 200 children and adults were enrolled in each country, and a second at the end of the study recruitment. This trial is registered with ClinicalTrials.gov, number NCT02758821.

Role of the funding source

The funders had no role in study design, data collection, data interpretation, or writing of the manuscript. The corresponding author (YL) had full access to all the data in the study and had the final responsibility to submit for publication.

Results

A total of 4116 patients were assessed for eligibility, of whom 2410 children and adults with a documented fever or a history of fever were prospectively recruited between June 8, 2016, and Aug 25, 2017, across ten facilities (figure 1). 803 patients were randomly assigned to CRP group A, 800 to CRP group B, and 807 to the control group (the intention-to-treat population). 598 patients in CRP group A, 593 in CRP group B, and 767 in the control group had follow-up data for both day 5 and day 14 and had been prescribed antibiotics in accordance with test results, so were included in the per-protocol population. Patient characteristics in the control group and the two intervention groups were similar at day 0 (table 2). Patients attended the facilities within a median of 2·5 days (IQR 1–4) after onset of symptoms. Clinically, respiratory symptoms were the most common presentation, followed by gastrointestinal and neurological symptoms (stratified results per country and age category are presented in the appendix).

The retrospective background surveys in the six Thai sites (6993 patients in total) showed that between 260 (29%) of 894 patients and 1406 (72%) of 1959 patients with a history of fever or a documented fever were prescribed antibiotics (figure 2). In patients with a documented fever, between 123 (37%) of 330 patients and 453 (87%) of 518 patients in each of the sites were prescribed an antibiotic; these proportions were largely unchanged in the 2 years preceding the study as was generally the case in a review of prescribing practices in all PCUs in the same district.[8] Among the four Myanmar sites (32 345 patients in total), only the Hlaing Tha Yar government hospital outpatient department had patient records that included febrile status and antibiotic prescription, in which 173 (69%) of 252 patients with a documented fever were prescribed an antibiotic. In the three MAM clinics, data were only available on the total number of non-routine visits (ie, excluding patients attending the clinic for HIV care, tuberculosis care, antenatal appointments, and family planning, as well as malnourished children), without a record of febrile status, and only the overall number of antibiotics prescribed during the corresponding period was known. Together, the pooled absolute numbers indicate that approximately 41% of non-routine clinic attendees received an antibiotic.

In the intention-to-treat population, we observed a significant difference in the trial primary outcome of antibiotic prescription from day 0 up to day 5 between the control group (318 [39%] of 807) and patients in group B (275 [34%] of 800), with a risk difference of –5·0 percentage points (95% CI –9·7 to –0·3) and an adjusted odds ratio (aOR) of 0·80 (95% CI 0·65 to 0·98; table 3). In group A,

	Control group	CRP group A	Risk difference (95% CI)	aOR* (95% CI)	CRP group B	Risk difference (95% CI)	aOR* (95% CI)
All age groups in Thailand and Myanmar							
Number of participants	807	803			800		
On day 0	297 (36·8%)	269 (33·5%)	−3·3 (−8·0 to 1·4)	0·86 (0·70 to 1·06)	245 (30·6%)	−6·2 (−10·8 to −1·6)	0·75 (0·60 to 0·92)
Between day 0 and day 5	318 (39·4%)	290 (36·1%)	−3·3 (−8·0 to 1·4)	0·86 (0·70 to 1·06)	275 (34·4%)	−5·0 (−9·7 to −0·3)	0·80 (0·65 to 0·98)
Between day 0 and day 14	323 (40·0%)	292 (36·4%)	−3·7 (−8·4 to 1·1)	0·85 (0·69 to 1·04)	279 (34·9%)	−5·2 (−10·0 to −0·4)	0·79 (0·64 to 0·98)
Patients aged <12 years in Thailand							
Number of participants	195	194			193		
On day 0	64 (32·8%)	56 (28·9%)	−4·0 (−13·1 to 5·2)	0·83 (0·53 to 1·28)	49 (25·4%)	−7·4 (−16·4 to 1·6)	0·68 (0·43 to 1·08)
Between day 0 and day 5	68 (34·9%)	61 (31·4%)	−3·4 (−12·8 to 5·9)	0·85 (0·55 to 1·31)	52 (26·9%)	−7·9 (−17·1 to 1·2)	0·68 (0·43 to 1·06)
Between day 0 and day 14	69 (35·4%)	61 (31·4%)	−3·9 (−13·3 to 5·4)	0·83 (0·54 to 1·28)	52 (26·9%)	−8·4 (−17·6 to 0·7)	0·66 (0·42 to 1·03)
Patients aged ≥12 years in Thailand							
Number of participants	201	200			199		
On day 0	63 (31·3%)	57 (28·5%)	−2·8 (−11·8 to 6·1)	0·86 (0·56 to 1·34)	65 (32·7%)	1·3 (−7·8 to 10·5)	1·06 (0·69 to 1·63)
Between day 0 and day 5	64 (31·8%)	60 (30·0%)	−1·8 (−10·9 to 7·2)	0·91 (0·59 to 1·40)	68 (34·2%)	2·3 (−6·9 to 11·5)	1·12 (0·73 to 1·71)
Between day 0 and day 14	64 (31·8%)	60 (30·0%)	−1·8 (−10·9 to 7·2)	0·91 (0·59 to 1·40)	69 (34·7%)	2·8 (−6·4 to 12·1)	1·14 (0·74 to 1·75)
Patients aged <12 years in Myanmar							
Number of participants	207	206			206		
On day 0	78 (37·7%)	77 (37·4%)	−0·3 (−9·6 to 9·0)	0·99 (0·66 to 1·48)	65 (31·6%)	−6·1 (−15·3 to 3·0)	0·76 (0·50 to 1·15)
Between day 0 and day 5	87 (42·0%)	84 (40·8%)	−1·3 (−10·8 to 8·3)	0·95 (0·64 to 1·41)	79 (38·4%)	−3·7 (−13·1 to 5·8)	0·86 (0·57 to 1·29)
Between day 0 and day 14	88 (42·5%)	86 (41·8%)	−0·8 (−10·3 to 8·8)	0·97 (0·66 to 1·44)	82 (39·8%)	−2·7 (−12·2 to 6·8)	0·90 (0·60 to 1·34)
Patients aged ≥12 years in Myanmar							
Number of participants	204	203			202		
On day 0	92 (45·1%)	79 (38·9%)	−6·2 (−15·8 to 3·4)	0·78 (0·52 to 1·15)	66 (32·7%)	−12·4 (−21·8 to −3·0)	0·58 (0·38 to 0·87)
Between day 0 and day 5	99 (48·5%)	85 (41·9%)	−6·7 (−16·3 to 2·3)	0·76 (0·52 to 1·13)	76 (37·6%)	−10·9 (−20·5 to −1·3)	0·63 (0·42 to 0·94)
Between day 0 and day 14	102 (50·0%)	85 (41·9%)	−8·1 (−17·8 to 1·5)	0·72 (0·49 to 1·07)	76 (37·6%)	−12·4 (−22·0 to −2·8)	0·59 (0·40 to 0·89)

The prescription of antibiotics from day 0 to day 5 is the primary outcome. Unadjusted odds ratios are presented in the appendix. Data are number or number (%) unless otherwise stated. aOR=adjusted odds ratio. CRP=C-reactive protein. *aORs were adjusted by site as a random effect.

Table 3: **Antibiotic prescription in the control group, group A (20 mg/L CRP threshold), and group B (40 mg/L CRP threshold)**

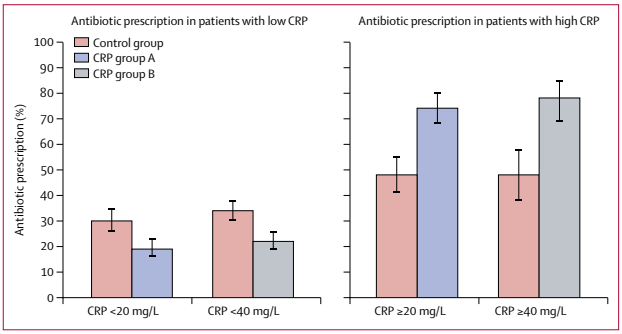

Figure 3: **Antibiotic prescription on day 0 in relation to the CRP thresholds in each of the intervention groups for all age categories and countries**
Error bars represent 95% CI. CRP=C-reactive protein.

290 (36%) of 803 patients were prescribed an antibiotic from day 0 to day 5, a non-significant difference compared with control group (risk difference −3·3 percentage points, 95% CI −8·0 to 1·4; aOR 0·86, 95% CI 0·70 to 1·06). The per-protocol analysis (appendix) showed a −20·4 percentage point difference (95% CI −25 to −15·7) in antibiotic prescription for the primary outcome in group B compared with the control group (aOR 0·35, 95% CI 0·27 to 0·45) and a −12·3 percentage point difference (−17·3 to −7·4) in group A compared with the control group (aOR 0·56, 0·44 to 0·71).

The trial primary outcome also included antibiotic prescription in relation to the CRP thresholds, with a higher proportion of patients with elevated CRP concentrations being prescribed an antibiotic in the intervention groups than in the control group (CRP ≥20 mg/L: 153 [74%] of 206 patients in group A *vs* 103 [48%] of 214 in the control group, p<0·0001; CRP ≥40 mg/L: 92 [78%] of 118 patients in group B *vs* 51 [48%] of 107 in the control group, p<0·0001; figure 3). Conversely, in patients with low CRP concentrations, antibiotic prescription was lower in the intervention groups than in the control group (CRP <20 mg/L: 119 [20%] of 595 patients in group A *vs* 134 [30%] of 445 in the control group, p<0·0001; CRP <40 mg/L: 153 [22%] of 682 patients in group B *vs* 186 [34%] of 552 in the control group, p<0·0001). Considering a threshold of 20 mg/L CRP as indicative of requiring antibiotics, 414 (63%) of 659 patients in the control group had an antibiotic correctly prescribed or

	Control group (n=807)	CRP group A (n=803)	Risk difference (95% CI)	aOR* (95% CI)	CRP group B (n=800)	Risk difference (95% CI)	aOR* (95% CI)
Neurological presentation							
Number of participants	210	195	..	..	195	..	..
On day 0	83 (40%)	71 (36%)	−3·1% (−12·6 to 6·3)	0·85 (0·56 to 1·29)	65 (33%)	−6·2% (−15·6 to 3·2)	0·71 (0·46 to 1·10)
Between day 0 and day 5	90 (43%)	78 (40%)	−2·9% (−12·5 to 6·7)	0·87 (0·58 to 1·31)	69 (35%)	−7·5% (−17·0 to 2·0)	0·68 (0·45 to 1·05)
Between day 0 and day 14	92 (44%)	78 (40%)	−3·8% (−13·4 to 5·8)	0·84 (0·55 to 1·26)	69 (35%)	−8·4% (−17·9 to 1·1)	0·65 (0·43 to 1·00)
Respiratory presentation							
Number of participants	649	630	..	..	626	..	..
On day 0	263 (41%)	218 (35%)	−5·9% (−11·2 to −0·6)	0·79 (0·62 to 0·99)	192 (31%)	−9·9% (−15·1 to −4·6)	0·63 (0·50 to 0·81)
Between day 0 and day 5	281 (43%)	237 (38%)	−5·7% (−11·1 to −0·3)	0·80 (0·63 to 1·00)	221 (35 %)	−8·0% (−13·3 to −2·7)	0·70 (0·55 to 0·89)
Between day 0 and day 14	284 (44%)	238 (38%)	−6·0% (−11·4 to −0·6)	0·79 (0·62 to 0·99)	225 (36%)	−7·8% (−13·2 to −2·5)	0·71 (0·56 to 0·90)
Gastrointestinal presentation							
Number of participants	199	207	..	..	177	..	..
On day 0	65 (33%)	75 (36%)	3·6% (−5·7 to 12·8)	1·17 (0·78 to 1·77)	63 (36%)	2·9% (−6·7 to 12·5)	1·16 (0·75 to 1·80)
Between day 0 and day 5	74 (37%)	82 (40%)	2·4% (−7·0 to 11·9)	1·11 (0·74 to 1·66)	67 (38%)	0·6% (−9·1 to 10·5)	1·04 (0·68 to 1·60)
Between day 0 and day 14	74 (37%)	83 (40%)	2·9% (−6·6 to 12·4)	1·13 (0·76 to 1·69)	67 (38%)	0·7% (−1·1 to 10·5)	1·04 (0·68 to 1·60)
Documented fever							
Number of participants	355	346	..	..	371	..	..
On day 0	165 (47%)	148 (41%)	−3·7% (−11·1 to 3·7)	0·85 (0·63 to 1·15)	141 (38%)	−8·5% (−15·8 to −1·3)	0·66 (0·49 to 0·90)
Between day 0 and day 5	173 (49%)	156 (45%)	−3·7% (−11·0 to 3·7)	0·85 (0·63 to 1·15)	153 (41%)	−7·5% (−14·7 to −0·3)	0·71 (0·52 to 0·96)
Between day 0 and day 14	175 (49%)	157 (45%)	−3·9% (−11·3 to 3·5)	0·84 (0·62 to 1·14)	154 (41%)	−7·8% (−15·0 to −0·6)	0·70 (0·51 to 0·95)

Data are number or number (%) unless otherwise stated. The prescription of antibiotics from day 0 to day 5 is the primary outcome. aOR=adjusted odds ratio. CRP=C-reactive protein. *aORs were adjusted by site as a random effect.

Table 4: **Subgroup analysis for antibiotic prescription**

withheld, as compared with 632 (79%) of 801 in group A. Similarly, assuming a threshold of 40 mg/L as indicative of the need for an antibiotic, 417 (63%) of 659 in the control group had an antibiotic appropriately prescribed or withheld, as compared with 621 (78%) of 800 in group B. Therefore, compliance with the test result was similarly high in the two intervention groups.

The risk difference in prescription in the subgroup of patients presenting with a documented fever was –7·5 percentage points (95% CI –14·7 to –0·3) in group B compared with the control group, and –3·7 percentage points (–11·0 to 3·7) in group A compared with the control group (table 4). Patients presenting with a respiratory syndrome in both intervention groups showed a significant reduction in antibiotic prescription, but did not show a significant reduction in the subgroup of patients with a neurological syndrome was observed. For patients with gastrointestinal symptoms, we did not observe a significant increase in prescription in the intervention groups compared with the control group.

Considering the follow-up period between day 0 and day 14, antibiotic prescription was –3·7 percentage points (95% CI –8·4 to 1·1) in group A, and –5·2 percentage points (95% CI –10·0 to –0·4) in group B, compared with the control group (table 3).

A post-hoc analysis showed that the prescription of broad-spectrum antibiotics (appendix) was lower at enrolment and throughout the follow-up visits in group B than in the control group (aOR 0·44, 95% CI 0·26–0·72 at day 0; aOR 0·79, 95% CI 0·64–0·97 from day 0 to day 5; and aOR 0·79, 95% CI 0·64–0·98 from day 0 to day 14), whereas this reduction was only significant on day 0 in group A (aOR 0·60, 95% CI 0·38–0·96).

We observed no differences in the prevalence of recovery as defined by patient declaration at day 5 or day 14 of follow-up between the study groups, with 495 (65%) of 764 at day 5 and 718 (94%) of 760 at day 14 in the 20 mg/L CRP group reporting recovery compared with 488 (63%) of 769 at day 5 and 733 (94%) of 779 at day 14 in the 40 mg/L CRP group, and 491 (64%) of 767 at day 5 and 738 (96%) of 772 at day 14 in the control group (appendix). We present a comparison of the time-to-event curves for recovery between groups A and B and the control group (figure 4). The corresponding log-rank test and HRs in group A and group B were non-significant. Similarly, no differences were found in these outcomes in the per-protocol analysis (appendix).

In cases of persistent illness at day 5 and day 14 of follow-up, the median symptom severity score corresponded to the mildest grade (ie, a severity score of 1 in a scale ranging from 1 to 4) and did not differ across the groups (appendix). The proportion of patients who attended unscheduled visits was 16 (2%) of 807 in the control group, 13 (2%) of 803 in group A, and 22 (3%) of 800 in group B with no significant difference between groups. CRP on day 5 was sufficiently elevated above the predefined threshold to notify health-care providers (50 mg/L in children and 100 mg/L in adults) in

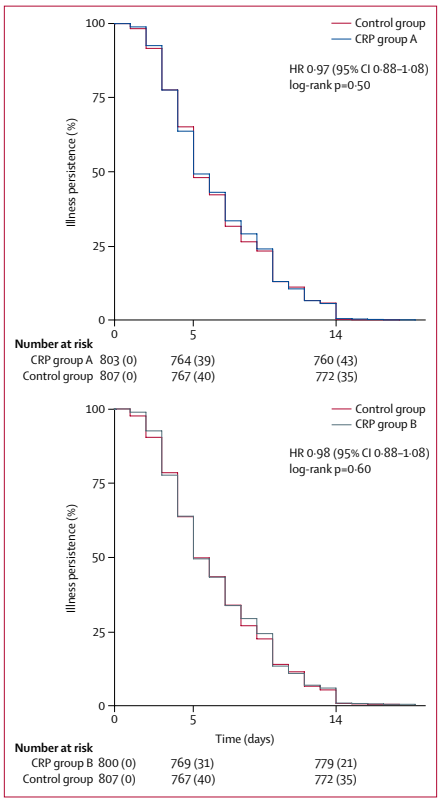

Figure 4: **Kaplan-Meier curves of symptom duration in the control group versus group A (20 mg/L) and group B (40 mg/L)**

eight (1%) of 706 patients in the control group, eight (1%) of 706 in CRP group A, and six (1%) of 726 in CRP group B with no significant difference between study groups. 74 (3%) of 2150 patients had a tympanic temperature of more than 37·5°C on day 5 and 31 (2%) of 1951 on day 14 with no significant differences between the study groups. Further details on clinical outcomes by age and country are presented in the appendix.

Among the 2410 study participants, 24 severe adverse events occurred (23 hospital admissions and one death), with ten (1%) severe adverse events in group A, 11 (1%) in group B, and three (<1%) in the control group (p=0·064 for group A and p=0·043 for group B compared with the control group). Among these 24 severe adverse events, 23 were classified as being not related to the study (nine in group A, 11 in group B, and three in the control group), and one was classified as being possibly related, occurring in a woman aged 25 years who had an abdominal complaint and fever with low CRP (randomly assigned to group A), initially diagnosed by the attending health-care provider as a hypersensitivity reaction, and no antibiotic was prescribed. During admission the woman was diagnosed with mesenteric lymphadenitis, and was prescribed antibiotics and discharged alive. No microbiological investigations were done at the hospital. One death occurred during the study, which was that of a man aged 78 years who was randomly assigned to group A and had chronic obstructive pulmonary disease and heart disease. The man's CRP concentration was high, and antibiotics were prescribed on day 0; this severe adverse event was classified as not related to the study.

Discussion

In this trial we have shown that, in primary care in southeast Asia, CRP testing with a threshold of 40 mg/L reduced antibiotic prescription in patients with a fever with no evidence of a difference in clinical recovery. Reductions in prescription were greater in patients with a documented fever, in those with respiratory presentation, and for broad-spectrum antibiotics. In addition to reducing antibiotic prescription, patients in the intervention groups with high CRP concentrations were more likely to be prescribed an antibiotic and those with low CRP concentrations were more likely to have antibiotics withheld than patients in the control group. These effects were more pronounced within group B, in which a higher threshold of 40 mg/L was used, whereas the differences between group A and the control group were of lesser magnitude and mostly not of statistical significance.

The effect size was smaller than anticipated when comparing the intervention and control groups but the difference in the proportion of patients who were prescribed antibiotics seemed to be much larger when comparing our trial data with our retrospective 2015–16 antibiotic prescription data; whether these reductions in prescribing, small or large, can have a real effect on mitigating drug pressure and the development of antimicrobial resistance is difficult to establish. With the majority of human antibiotic consumption occurring in the community and in patients with fevers and respiratory illness in particular, even small reductions in prescription could imply a large alleviation of drug pressure. Further modelling and cost-effectiveness analyses are required to explore whether these reductions and the cost of achieving them are warranted from an economic and global health perspective. A modelling analysis conservatively estimated that in the Thai context the economic costs of antimicrobial resistance per course of broad-spectrum penicillins (widely used in this study) was approximately $10. A reduction of just 10 percentage points from baseline with the use of a CRP test costing $1 could therefore be considered cost beneficial from a societal perspective.[29]

The risk of serious bacterial infection in patients in primary care without clear clinical danger signs is low,

and the majority of patients in the trial had low CRP concentrations on day 0 (<20 mg/L in 72% of patients and <40 mg/L in 86%; appendix). As CRP is known to be of high sensitivity but only moderate specificity in the detection of bacterial infections, the higher threshold of 40 mg/L is likely to be appropriate for these settings, and stricter adherence to test results at this threshold would generate larger reductions in prescribing, as shown in the per-protocol analysis (>20% reduction for the primary outcome between group B and the control group; appendix). This reduction in prescribing might be achieved in scale-up programmes driven by local health authorities supported by high-level policy change. Integrating CRP testing into clinical guidelines could also enhance prescribers' confidence, as might electronic user-friendly algorithms that strengthen the consistency of fever management.[30] Clinical and communication skills training could also enhance compliance with these algorithms.[31]

A systematic review of studies on biomarker testing in febrile children in emergency departments, all in high-income settings, concluded that CRP testing could be diagnostically useful, recommending a low threshold of 5 mg/L to rule out serious infection and a threshold of 80 mg/L to suggest the presence of serious infection.[32] A cluster randomised trial in Belgium concluded that CRP testing in primary care should be targeted at children at high risk of severe infection after clinical assessment.[33] This targeting could also avoid medicalisation of minor ailments. A multicountry European study showed enhanced pneumonia prediction in patients in primary care presenting with acute cough when CRP testing was added to a clinical algorithm based on symptoms alone.[34] This risk stratification relied on the availability of experienced clinicians. In Thailand, which has a well-developed public health sector, such strategies could be relevant, with adequate supervision of health-care providers' clinical skills combined with clear referral and follow-up systems to ensure patient safety. The low number of prescriptions in the Thai control group suggests that reductions in prescribing could be attained with better training, supervision, or financial incentives, with CRP testing offering modest additional gains.

The evidence base for the evaluation of the effect of CRP-guided treatment from lower-income settings is small, and, because of particular comorbidities and pathogen exposure in lower-income countries in the tropics, data from high-income (non-tropical) settings are not easily comparable. A Tanzanian-based clinical trial on fever management showed that a host of interventions including CRP and procalcitonin tests, pulse oximetry, haemoglobin tests, and an electronic patient management algorithm, were able in combination to reduce prescribing from 95% to approximately 10% of patients.[30] The costs and benefits of implementing a more comprehensive bundle of interventions need to be weighed against the lower costs and benefits of CRP testing alone. A trial using only CRP tests in acute respiratory tract infections in primary care in Vietnam reported a 20% reduction in prescribing on first attendance.[21]

Data for the retrospective survey in Thailand were obtained from a search of routinely collected electronic medical records, making the verification of the data challenging; however, CRF data from a subsample of patients included in this retrospective survey were compared with their respective routine medical records and were found to be consistent.[8] The retrospectively collected 2015–16 antibiotic prescription data for the MAM sites did not include patients' febrile status and only had the total number of prescriptions in the corresponding months, therefore our retrospectively collected prescription data are likely to underestimate the actual proportion of patients with a suspected infection who were prescribed an antibiotic. Despite these limitations, the retrospective surveys indicate much higher prescription rates than those observed in the research environment.

The primary outcome of the study, antibiotic prescription in response to CRP testing, is behavioural rather than biological, and therefore results need careful interpretation that considers contextual factors such as the broader policy environment and study design biases. In Thailand, reductions in prescribing were probably driven by policy changes implemented during the study period. Although our study adopted a mixed-methods approach that included social research components to better understand these contextual factors,[35] disentangling and quantifying their relative contribution is challenging. The addition of a diagnostic tool did, however, enable further reductions in prescribing, supporting a multifaceted approach.

The reduction in prescribing in all study groups could also be explained by the Hawthorne effect because of the presence of research staff on site who were carrying out the CRP tests and monitoring prescribing. Prescribing in the control group might also have been affected by contamination (because of recognition that most patients have low CRP concentrations and do not require an antibiotic), therefore underestimating the effect that CRP testing could have in a routine care environment. The ideal study design would therefore have been a cluster randomised trial without the presence of research staff on site and with the tests being performed by routine health-care providers.

Our study was powered to detect a difference in antibiotic prescription, rather than clinical outcomes. The reason we chose this endpoint was that CRP is already widely used in hospital settings in the management of febrile patients[32] and has been shown to be highly sensitive in detecting bacterial infections in the region.[18] In the context of primary care and with the exclusion of patients presenting with clear danger signs, we expected very few severe outcomes, as was indeed the

case. Although powering the study to detect differences in such rare events would not be feasible, the study recruited more than 2400 patients with high follow-up and no evidence of a difference between the groups was detected in the extensive measures of clinical outcome. Furthermore, in our study CRP tests were provided as an aid to health-care providers' clinical judgment, with only soft recommendations on how the tests could inform their prescribing decisions.

CRP testing in a southeast Asian primary care setting has previously been shown to reduce antibiotic prescribing in patients with respiratory tract infection and our trial extends this evidence base to those with a fever. Together, these patients represent the majority of acute primary care attendees. The fact that the proportion of patients in the control group who were prescribed an antibiotic seemed to be lower than our retrospectively collected data on antibiotic prescribing in the region indicates that substantial progress is attainable through other mechanisms, with CRP testing using a threshold of 40 mg/L bringing a modest incremental effect. These findings correspond with previous studies recommending a restricted use of CRP tests in high-risk groups, including children with high fever. This restricted use might well be viable in facilities staffed by experienced clinicians and where patient follow-up is feasible. In more peripheral settings, with minimally trained health-care providers and where follow-up of patients is a challenge, testing all febrile patients could provide an additional safety net, ensuring that patients requiring treatment are identified as such. In these settings, low-cost lateral flow devices are most likely to be relevant, for which we provide further evidence on the optimal threshold. Introduction of the tests should be planned in consideration of the local policy environment and accompanied by training for health-care providers and education for patients on the ability of CRP testing to identify when antibiotic treatment is required, and the need for better targeting of antibiotics in the fight against antimicrobial resistance.

Contributors

TA, RCG, MMMS, NS, SD, JT, ENC, MJH, TW, DL, ND, FS, and YL contributed to the literature review. The study was designed by TA, RCG, TW, ND, FS, SN, DI, SB, ZD, NW, HMT, HW, PWS, and YL. Data collection was done by MMMS, JC, NNT, JH, PS, ENC, PWS, and MJH, data analysis by TA, RCG, MMMS, MM, TW, and YL, and data interpretation by TA, RCG, MMMS, SD, MM, ENC, MJH, TW, DL, FS, and YL. Figures were created by TA, RCG, MMMS, DL, and YL. The manuscript was written by TA, RCG, and YL. All authors reviewed the manuscript, added appropriate revisions, agreed to submission for publication, and approved the final version. TA and YL are guarantors.

Declaration of interests

We declare no competing interests.

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