UNIVERSITY OF CAPE COAST

SERUM BIOMARKERS DISTINGUISHING MALARIA FROM NON-MALARIA FEVER IN PAEDIATRIC PATIENTS IN BLANTYRE, MALAWI

ZEFANIAH JOEL KATUAH

2024

UNIVERSITY OF CAPE COAST

SERUM BIOMARKERS DISTINGUISHING MALARIA FROM NON-MALARIA FEVER IN PAEDIATRIC PATIENTS IN BLANTYRE, MALAWI

BY

ZEFANIAH JOEL KATUAH

Thesis submitted to the Department of Microbiology and Immunology of the School of Medical Sciences, College of Health and Allied Sciences at the University of Cape Coast in partial fulfilment of the requirement for the award of a Master of Philosophy Degree in Infection and Immunity.

AUGUST 2024

DECLARATION

Candidate's Declaration

I so declare that this thesis is the product of my own unique study and that no portion of it has been submitted, either at this university or elsewhere, for consideration for another degree.

Candidate's Signature _____ Date: 28th April, 2025

Name: Zefaniah Joel Katuah

Supervisor's Declaration

We hereby declare that the preparation and presentation of this thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature _____ Date: 29th March, 2025

Name: Dr Roland Osei Saahene

Co- Supervisor's Signature _____ Date: 02nd April, 2025

Name: Prof Samuel Victor Nuvor

Co- Supervisor's Signature _____ Date: 28th April, 2025

Name: Associate Professor Tonney Nyirenda

ABSTRACT

Identifying the aetiology of fever is challenging in paediatric patients, particularly in resource-limited settings like Malawi. Most infectious diseases, including malaria, present with overlapping clinical signs associated with fever. This study aimed to identify specific serum biomarkers that differentiate malaria from non-malarial fever in paediatric patients in Blantyre, Malawi. Ninety archived serum samples obtained from paediatric patients who presented with fever were grouped into non-malarial (n=25), malarial (n=46) and healthy controls (n=19) based on temperature and mRDT results. Serum samples were used to measure the concentration of CRP, ICAM-1, IL -6, IL-1β, IL -10, Ang-2 and vWF-1 biomarkers using Luminex xMAP® technology. The biomarker and haematology results obtained from participants' records were analysed using R statistics software. Malaria cases presented with a higher temperature (p=0.0089), low platelet counts (p=0.02) and haemoglobin levels (p=0.01) compared to non-malarial fever. Malarial fever had elevated IL-6 (AUROC=0.66, p= 0.04), IL-10 (AUROC = 0.81, p= ≤0.0001) and CRP (AUROC=0.60, p=0.09) compared to non-malarial fever and controls. Non-malarial fever had elevated IL-1β (AUROC =0.9, p=0.02), ICAM-1 (AUROC= 0.76, p= <0.05), Ang -2 (AUROC=0.79, p=0.005), vWF-1 (AUROC=0.71, p ≤ 0.001) compared to malaria and controls. IL-10 and IL-1β correctly differentiate malarial fever from non-malarial fever with IL-10 levels increased in malarial fever and IL-1β increased in non-malarial fever. These biomarkers can significantly enhance diagnostic accuracy and treatment outcomes in resource-limited settings and can be effectively utilized in point-of-care testing.

KEYWORDS

Malarial fever

Non-malaria fever

Co-infections

Paediatric patients

Biomarkers

Sepsis

Gold standard

Biomarker analysis

Inflammation

Machine learning

ACKNOWLEDGEMENTS

I am sincerely grateful to my principal supervisor Dr Roland Saahene and my co-supervisor Professor Samuel Victor Nuvor and Associate Professor Tonney Nyirenda for their guidance and encouragement throughout this research journey. Their insightful feedback and unwavering support have significantly contributed to the completion of this thesis. Furthermore, I would like to thank Associate Professor Tonney Nyirenda for allowing me to use his laboratory and samples. I would also like to thank my family and friends for their patience, understanding and constant encouragement during this challenging but rewarding endeavour.

DEDICATION

I dedicate this thesis to myself and my future babies.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS

CRP…….. C - reactive protein

ICAM-1…… Intercellular Adhesion Molecule-1

IL-6……... Interleukin-6

IL-1β …… Interleukin-1 Beta

IL-10……...Interleukin-10

ANG-2……Angiopoietin-2

vWF-1…….von Willebrand Factor-1

TNF-α……..Tumor Necrosis Factor Alpha

IFN-γ……Interferon Gamma

WHO……World Health Organization

RDT…….Rapid Diagnostic Test

PCR…….Polymerase Chain Reaction

ELISA……. Enzyme-Linked Immunosorbent Assay

CNS……..Central Nervous System

HRP2……Histidine-Rich Protein 2

RBC……..Red Blood Cell

WBC…….White Blood Cell

G6PD…….Glucose-6-Phosphate Dehydrogenase

Hb……...Haemoglobin

CHAPTER ONE

INTRODUCTION

This chapter gives a general overview of the study by providing background information, problem statement, objectives and study limitations. This study aimed at identifying serum biomarkers that differentiate malaria from non-malaria fever in Malawian paediatric patients. The rationale is to improve diagnostic accuracy of causes of fever, thereby enabling timely and targeted interventions and potentially reducing mortality rates.

Background to the study

Malaria continues to be one of the most prevalent infectious diseases affecting human health, mainly in the tropics (Obeng-Aboagye et al., 2023). It is estimated that malaria cause death of 435,000 lives annually with Africa topping the table. The protozoan Plasmodium falciparum is the prevalent cause of malaria (Doumbe-Belisse et al., 2021). It is spread to humans when female Anopheles mosquitoes feed on human blood through bites (Caminade et al., 2019).

Recently, there has been a remarkable progress in malaria control. Despite such strides, the disease remains a major global health problem. The estimated cases of malaria were 247 million and 409,000 deaths were reported in 2021 pooled data from 84 malaria-endemic countries (*The 2023 WHO World Malaria Report - The Lancet Microbe*, n.d.). In 2021, approximately 96% of malaria deaths and 95% of all cases occurred in the WHO-Africa area alone with 80% of deaths occurring in children under 5 years (Venkatesan, 2024).

Five known species of plasmodium parasites cause malaria in humans. Most of the more severe and deadly cases affect pregnant women and children under 5 years (Danis, 2023). In sub-Saharan Africa, *Plasmodium falciparum* is the most common parasite while *Plasmodium vivax* is the dominant parasite in Asia and causes significant morbidity. Less frequently occurring parasites that rarely cause severe disease are *Plasmodium ovale* and *Plasmodium malariae* (Millar & Cox-Singh, 2015; Singh & Daneshvar, 2013).

Malawi is found in the southern eastern region of Africa and lies within a region endemic to malaria and other neglected tropical diseases. The World Health Organisation report of 2021 indicated that Malawi has a child mortality rate of 41.9 deaths in every 1,000 live births. At Kamuzu Central Hospital, a major referral hospital in Malawi, 743 paediatric deaths in that year were recorded in a study looking at acute and inpatient care (Fitzgerald et al., 2024). In the same study, it was reported that the mortality rate from infectious diseases in Malawi ranged monthly from 2.2% to 4.4%. Sepsis, HIV-related diseases, malnutrition and malaria were the leading causes of mortality. The most common causes of death in paediatrics are sepsis, infection of the lower respiratory, acute gastroenteritis, malaria and meningitis. It was also noted that *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus aureus* were the commonly isolated bacteria in paediatric fever cases (Escadafal et al., 2020).

An increase in body temperature above the typical daily range is what defines a fever (Baltzell et al., 2019). Children under 5 years present with fever due to a lot of health issues and it accounts for 15–25% of hospital visits in primary care (Dorado et al., 2024; Weghorst et al., 2022; Whitburn et al., 2011). In children younger than five years old, fever denotes systemic inflammation,

usually caused by a bacterial, viral, parasitic, or less frequently, a non-infectious aetiology (Herlihy et al., 2016) In malaria-endemic regions, including Malawi, fever is thought to be caused by malaria and is treated as such, excluding the possibility of other causes including viruses and bacteria.

Fever is one of the most prevalent symptoms in children and its similar clinical characteristics pose a challenge to clinicians due to the vast range of possible aetiologies (Madut et al., 2021). It is challenging to implement diagnostic strategies that target the potential causes of fever and for many patients, the causative organism remains unknown. Determining the real source of fever is still a challenge in many situations where numerous co-infecting organisms are present. Fever is mostly caused by viral and bacterial bloodstream infections (Babigumira et al., 2017).Antimicrobial resistance has become more common in bacterial isolates in recent times, especially with the spread of *Salmonella enterica* which is resistant to fluoroquinolones and extended-spectrum β-lactamase-producing Enterobacteriaceae (Kalumbi et al., n.d.). This can be attributed to improper use of medications due to misdiagnosis and un-rational use by safe medication (Essack et al., 2017; Pokharel et al., 2020).

Malaria caused by *Plasmodium falciparum* revolves between female anopheline mosquito and humans. During a blood meal, sporozoites are injected into human blood. Firstly, the sporozoites infect liver cells, where they proliferate in an asymptomatic replication cycle. Hepatocytes release invasive merozoites, which infect red blood cells and where they repeatedly proliferate, replicate, emigrate and invade new RBCs. Fever, headaches and exhaustion are among the symptoms that arise at this stage of erythrocyte infection, which is a

symptomatic stage (Mawson, 2013; Shahbodaghi & Rathjen, 2022; Walker & Rogerson, 2023)

Malaria mainly presents as an uncomplicated febrile illness associated with fever, vomiting, general body pains and headache which resolves within a short time (Frimpong et al., 2022; Obeng-Aboagye et al., 2023). On the other hand, malaria advances to a severe and sometimes fatal illness in 1–2% of cases. Age, exposure and immunological state are a few factors that could affect this progression (Wassmer et al., 2015). Children under 5 years old can develop serious malaria infection due to their immune system which is still developing.

Fever is a condition associated with a temperature of ≥37.5 °C and can be grouped as fever with or without origin. A fever with a known origin is one with an identified cause and a fever with an unknown origin is the one with no known cause. Fever with unknown origin is commonly caused by viral and bacterial infections and sometimes autoimmune inflammatory diseases (Dayal & Agarwal, 2016). In endemic regions such as Malawi, limited resources force healthcare workers to issue fever diagnosis based on symptoms such as fever longevity, headache, nausea, vomiting, dizziness, joint pain and a patient's history of malaria (Mandala et al., 2022; Munyenyembe et al., 2018).

In a study of Asian adults, it was established that shivering, fever and sweating are shown to be less significant in malaria diagnosis because other infectious diseases present with the same (Bria et al., 2021). In a related study in Sierra Leone, it was found that a lot of people were doing self-diagnosis of fever related diseases and there was poor hospital-seeking behaviour. In a total of 882 households that were home to 5410 individuals, 41% of the 910 people who reported having symptoms that the household believed to be malaria in the

previous month had a similar diagnosis made by a medical expert or a laboratory test (Ansumana et al., 2013; Thomson et al., 2011). This demonstrates the destructive action of diagnosing fever by clinical symptoms.

Early and accurate diagnosis is paramount for ensuring prompt and effective treatment of all kinds of infectious diseases. Assessment of fever and pinpointing the causative agent is challenged by the lack of proper guidelines in Saharan Africa (Birhanu et al., 2016; Mandala et al., 2022). In paediatric patients, diagnosis becomes even more challenging due to the non-specific clinical presentation that is often observed. Paediatric patients frequently present with fever and a range of non-specific symptoms that can be difficult to differentiate among infectious diseases (*World Health Organization (WHO)*, n.d.) This makes it arduous for healthcare providers to pinpoint the exact cause of the fever.

In Malawi, where malaria is endemic and co-infections with other pathogens are common, the diagnostic dilemma is escalated. Children under the age of five suffer mostly from bacterial infections, with *Streptococcus* (GBS), *Staphylococcus aureus* and *Streptococcus pneumoniae* being the main culprits (Okomo et al., 2019). Diagnostic methods such as light microscopy and Malaria Rapid Diagnostic Tests (mRDT) are limited in their capacity to identify and differentiate coinfections leading to misdiagnosis or delayed diagnosis (Calderaro et al., 2024). Misdiagnosis and delayed diagnosis are associated with several adverse outcomes, including inadequate treatment, disease progression and even death. Paediatric patients are vulnerable to these consequences due to their developing immune system and limited capacity to communicate their symptoms effectively (Wambani & Okoth, 2022).It is therefore imperative to

develop innovative diagnostic markers that can enhance the precision of distinguishing between malaria and non-malaria fever in paediatric.

The importance of accurately and promptly diagnosing malaria and its differentiation from other feverish conditions is unquestionably necessary. The need is escalated primarily in situations where normal Rapid Diagnostic Tests (RDTs) are unreliable (Calderaro et al., 2024). Microscopy of blood smears is still on the cutting edge of diagnosing malaria and is regarded as the gold standard procedure, however, it requires training and personal skills to achieve quality results (Cohen et al., 2020; Varo et al., 2020; Zhang et al., 2024).

Rapid diagnostic tests (RDTs) are used to diagnose malaria; however, the validity of these tests is jeopardised by mutations in the target protein, the histidine-rich *Plasmodium falciparum* protein (Feleke et al., 2021). Low parasite counts can also result in decreased test sensitivity. Once the sensitivity is low, malaria fever can be presumed to be a fever of either bacterial or viral origin. It is hard to rely on microscopic examination since it takes more time (Bisoffi et al., 2012). Diagnosis is delayed at most clinics, particularly in rural regions where there is a shortage of qualified microscopists, instead, samples are sent to state-of-the-art laboratories. If an epidemic breaks out, things might get really bad and thus finding new methods of diagnosing malaria is crucial.

At any stage of infection, the immune system produces proteins that act as markers of inflammation. Biomarkers are substances that could be detected, evaluated and employed as a sign of pathogenic stress, biological processes or a patient's reaction to a treatment (Bhardwaj & Baran, 2023). Biomarkers can be used to identify the organism causing an illness, distinguish it from non-infectious diseases, assign disease risk profiles, forecast results, help choose and

monitor treatment (Eggimann et al., 2019; Sridharan & Chamberlain, 2013; Stoma et al., 2017; van Engelen et al., 2018).

Prior research has indicated that proteins produced during infection may act as transcript signatures and markers that can be used to determine disease progression, prognosis and diagnosis of infectious diseases (Průcha et al., 2018; van Engelen et al., 2018). Disease identification and therapy follow-up can depend heavily on biomarkers. Diseases are usually treated with the highest chance of success when identified early. Unfortunately, the full promise of biomarkers is not being realised due to a variety of technical issues with existing technology for biomarker detection (Bodaghi et al., 2023). Biomarkers that can reliably identify malarial and non-malarial fever at an early stage using straightforward experimental techniques will therefore undoubtedly help to solve diagnostic challenges. Here, there is a lot of promise for the application of novel serum biomarkers in fever diagnosis.

Certain proteins like C-reactive protein (CRP), Intercellular Adhesion Molecule-1 (ICAM-1), Interleukin -6 (IL-6), Interleukin -10 (IL-10), Angiotensin -2 (Ang-2) and von Willeband Factor-1 (vWF-1) have shown potential in aiding the differential diagnosis of infectious and inflammatory conditions (Henriquez-Camacho & Losa, 2014). In the diagnosis and prognosis of complicated malaria, several biomarkers, including CRP, Ang- 2, angiopoietin-2/1 ratio, platelet levels and HDR- 2, have produced promising findings (Foko et al., 2022).

The most utilised laboratory parameters as indicators of paediatric fever are procalcitonin, CRP and Full Blood Count (FBC) (Cantey & Lee, 2021; Mathur & Behera, 2019; Weitkamp, 2021). Biomarkers can help diagnose

bacterial and other illnesses more accurately and help track the course of the infectious process. Though many novel biomarkers associated with infectious diseases have been studied, only a few have made it to the point where they may be applied in clinical settings (Foko et al., 2022). Although there are currently several biomarkers for therapeutic use in sepsis, their efficacy is frequently constrained by their low sensitivity and specificity (Weitkamp, 2021). An in-depth evaluation of biomarkers can have the potential to serve as reliable indicators for distinguishing between malaria fever and non-malarial fever, even in the presence of co-infections.

In areas where malaria is widespread, the creation of precise and effective diagnostic instruments based on biomarkers may completely change how paediatric patients with febrile diseases are treated. It would facilitate timely and appropriate treatment, reduce the risks associated with misdiagnosis and improve overall patient outcomes (Cantey & Lee, 2021; Weitkamp, 2021). Additionally, the research in this area lays the foundation for the creation of cost-effective and accessible diagnostic solutions that are well-suited for resource-limited settings, where the burden of infectious diseases like malaria is the highest. Thus, the pursuit of novel diagnostic markers in Malawian paediatric patients represents a critical step toward advancing healthcare and mitigating the region's experience with malaria and other fever diseases.

Statement of the problem

Malaria and other infections pose a significant public health challenge in Malawi, especially among children under 5 years (Mandala et al., 2022; Munyenyembe et al., 2018). Distinguishing malaria from other febrile illnesses

in paediatric cases is complicated by presentation with non-specific symptoms and co-infections that further confound clinical assessment. In many cases, any clinical presentation of fever is taken as malaria thereby overlooking the possibility of bacterial and viral infections (Madut et al., 2021). Current methods for diagnosing malaria, such as microscopy and rapid diagnostic tests, are not always reliable and have serious limitations.

The use of serum biomarkers can alleviate the problem by providing an alternative way of diagnosing and differentiating malaria and other infectious diseases. Such markers could facilitate a targeted and timely intervention, potentially reducing mortality rates associated with malaria and co-infections.

This current study aimed to identify serum biomarkers distinguishing malaria from non-malaria fever in Malawian paediatric patients. The study used C-reactive protein, ICAM-1, Interleukin -6, Interleukin -1B, Interleukin -10, ANG-2 and vWF-1 profiles to distinguish malaria and other infections. The findings could have a positive impact on health by enhancing more accurate and reducing delay in diagnosis of malaria and other infections in children, which could potentially reduce mortality rates.

Aims, objectives and research hypothesis

Aim of the study

The study aimed at exploring biomarker profiles that differentiate malarial fever from non-malarial fever in paediatrics in Blantyre, Malawi.

Specific objectives

1. To determine the biomarker profiles of CRP, ICAM-1, IL -6, IL-1 β, IL -10, Ang-2 and vWF-1 in pediatric patients with malaria fever to those with non-malarial fever.
2. To compare levels of biomarkers (CRP, ICAM-1, IL -6, IL-1 β, IL -10, Ang-2 and vWF-1) in paediatrics with malaria fever to those with non-malarial fever.
3. To distinguish between malaria fever and non-malaria fever using the identified biomarker profiles.
4. To assess the diagnostic accuracy of biomarker profiles (CRP, ICAM-1, IL -6, IL-1 β, IL -10, Ang-2 and vWF-1) in differentiating malarial fever from non-malarial fever

Research hypothesis

The study hypothesis was that profiles of CRP, ICAM-1, IL -6, IL-1 β, IL -10, Ang-2 and vWF-1 will be highly expressed in malarial fever and in non-malaria fever in paediatric patients in Blantyre.

Significance of the study

This study tackles a critical gap in understanding childhood fevers by exploring seven potential biomarkers expressed in fever either due to malaria or non-malarial fever. This study is significant in paediatric healthcare as it investigates the biomarker profiles of C-reactive protein, ICAM-1, Interleukin -6, Interleukin -1 β, Interleukin -10, Ang-2 and vWF-1 in paediatric patients to improve diagnostic precision for febrile illnesses. The comparison of biomarker levels in malaria and non-malarial fever contributes valuable data for

understanding pathophysiological processes and refining targeted diagnostic strategies.

The focus on differentiating malaria from non-malarial fever holds promise for the development of new diagnostic tools. Additionally, the assessment of diagnostic accuracy has direct implications for optimizing treatment interventions, preventing unnecessary antimalarial use and improving resource allocation in paediatric healthcare. In essence, this research has the potential to enhance diagnostic approaches, inform treatment decisions, and impact public health strategies in the paediatric population**.**

Delimitations

The study focuses specifically on paediatric patients and this limits the generalizability of its findings to other age groups. The biomarkers under investigation— C-reactive protein, ICAM-1, Interleukin -6, Interleukin -1 β, Interleukin -10, ANG-2 and vWF-1 —were chosen based on their relevance to paediatric febrile illnesses, potentially excluding other biomarkers that could be of significance in different contexts.

The study's geographical scope is Blantyre. The location can have effects on the generalizability of findings to broader geographic settings with different epidemiological profiles. The research also relied on the availability of certain laboratory resources and technologies such as the availability of equipment and variations in these resources across different settings could impact the reproducibility of the study.

While efforts are made to distinguish between malaria and non-malarial fever, the study acknowledges the potential influence of confounding factors

that might affect the accuracy of diagnostic categorization. The presence of autoimmune infections, other undetectable causes of fever, and co-infections with other pathogens can complicate the clinical picture and lead to misdiagnosis. Additionally, factors such as patient age, nutritional status, and underlying health conditions can impact immune response and fever presentation, further obscuring diagnostic clarity. The retrospective nature of the study design may introduce limitations in data availability and completeness, as historical records may lack comprehensive information on all relevant variables, including prior medical history and laboratory test results. Furthermore, variations in diagnostic criteria and practices across different healthcare settings could lead to inconsistencies in how fever cases are categorized.

Limitations

There are a lot of proteins that are produced in response to infection. The study only focused on a specific set of biomarkers and this may overlook other potentially relevant markers not included in the investigation. The generalizability of findings is constrained by the study's location, limiting the applicability of results to broader geographic settings with distinct epidemiological characteristics. Due to the unavailability of data on bacteriological and viral tests, all non-malarial fevers were not grouped into bacterial or viral. This can limit the understanding of the results since some markers can be elevated in viral and not in bacterial fevers. Additionally, variations in healthcare infrastructure and laboratory resources may impact the reproducibility of the study in different settings.

Definition of terms

1. Paediatric Patients: Individuals within the age range of infancy to adolescence, typically up to 5 years old.
2. Febrile Illnesses: Conditions characterized by fever, including but not limited to malaria, non-malarial fever, and co-infections.
3. Biomarkers: Molecules measurable in the body, indicating biological processes, pathogenic stress, or responses to therapy.
4. Protozoan: A group of single-celled microorganisms, including Plasmodium falciparum that cause malaria.
5. Endemic: Prevalent or regularly found in a particular geographic area.
6. Sensitivity: a diagnostic test's capacity to accurately identify those who have the illness.
7. Sepsis: an extreme, potentially fatal response to an infection that may result in organ failure.
8. Gold Standard: A diagnostic or treatment method widely accepted as the most effective

Organisation of the study

The study is organised into 5 chapters.

Chapter One: This chapter provides an introduction to the study, a summary of the goals, background and significance of the research.

Chapter Two: This chapter gives a thorough review of the literature. Much focus is on looking at pertinent research on the differentiation of malarial from non-malarial fever in paediatric patients.

Chapter Three: Chapter Three explains the methodology which provides detailed descriptions of research methods, including participant selection criteria and data collection processes for biomarker analysis.

Chapter Four: Presents findings on biomarker expression across different fever conditions.

Chapter Five: The last chapter contains a conclusion and summarises key findings, discusses their implications, and highlights the study's contributions.

CHAPTER TWO

LITERATURE REVIEW

Introduction

This chapter includes literature related to the diagnosis of malarial fever and non-malarial fever using biomarkers. Patterns and epidemiology of infectious diseases have been explored. Much focus has been put on exploring different markers that are associated with either malaria or non-malarial fever in paediatric patients.

Meaning and causes of fever

Fever is a naturally occurring adaptive mechanism and forms part of the immune system's inflammatory response. It is characterised by an unnatural rise in body temperature that takes place in response to certain physiological alterations regulated and mediated by the Central Nervous System (CNS) (Bakalli et al., 2022; Madut et al., 2021). Herlihy et al. (2016) indicated that fever is interpreted as a temperature of 38 degrees Celsius (rectal or tympanic), 37.5 degrees Celsius (oral) or 37.5 degrees Celsius (axillary). This fever response aims to provide an unfavourable environment for the infecting pathogen and hence aid in its clearance.

Paediatricians and other healthcare professionals treat fever as one of the most prevalent clinical presentations in paediatrics (Babigumira et al., 2017). Fever can be with a known origin or with an unknown origin. Fever with a temperature higher than 38.3 degrees Celsius for longer than three weeks or the one that does not improve after one week of inpatient testing is considered fever of unknown origin(Dayal & Agarwal, 2016). Fever in paediatrics can be

due to viral, bacterial, parasitic, or less commonly non-infectious causes (Bakalli et al., 2022; Herlihy et al., 2016). Studies in infectious disease have demonstrated that salmonellosis, tuberculosis, malaria, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* are the common causes of fever (Dayal & Agarwal, 2016; Orimadegun et al., 2022). Even though most fevers are viral in origin, there has been less attention to viral causes of fever (Bakalli et al., 2022). This can be attributed to inadequate testing mechanisms for viral infections. Fever in younger patients makes up 15–25% of consultations in emergency rooms and primary care clinics (Weghorst et al., 2022; Weitkamp, 2021; Whitburn et al., 2011). Therefore, it is crucial to quickly make decisions on the diagnosis of the aetiology of fever and hence issue treatment.

The burden of fever-related illnesses in children

Infectious diseases mostly present with fever. Overall, infections account for 26.5% of the worldwide burden of disease and mortality (Adedokun & Yaya, 2020). In paediatrics, 25% of all deaths worldwide are because of infections. The clinical manifestations of these infections vary from minimal symptoms such as fever and headache to sepsis which presents with multiple organ failure and death (Minderhoud et al., 2020; Rudd et al., 2020; Vincent, 2020). Six infectious diseases—lower respiratory infections, diarrhoea, malaria, meningitis, whooping cough and STI —are identified in the Global Burden of Disease (2019) report as the top causes of hospitalisation and mortality in paediatrics. These illnesses all frequently manifest with fever.

Studies done at Kamuzu Central Hospital in Malawi and other research institutions consistently identify malaria, pneumonia/bronchiolitis, sepsis, and diarrheal diseases as primary contributors to childhood mortality (Connon et al., 2021; Fitzgerald et al., 2024). Fitzgerald *et al*. (2018) results are consistent with data from the World Health Organisation (2021) that there is a high rate of fever-related mortality indicated at 41.9 fatalities per 1000 live births in Malawi. Despite these findings, there remains a lack of consensus on rapid and accurate diagnostic tools that could potentially improve outcomes by enabling timely and targeted diagnosis of fever-presenting diseases.

Escadafal et al. (2020) agree with Fitzgerald et al. (2024) by demonstrating that sepsis, lower respiratory tract infections, acute gastroenteritis, malaria and meningitis are the top five causes of death in childhood. In all these studies, the causes of clinical illness present with fever and this brings in a diagnosis challenge. These studies did not pinpoint a diagnostic measure that can be used to quickly identify the cause of fever and prevent disease deterioration.

Overview of malaria

Malaria parasite transmission, epidemiology and life cycle

Malarial fever has been documented in Egyptian and Chinese texts dating back to 2700 BC (Garcia, 2010). According to World Health Organisation reports, over 249 million people globally are afflicted with parasites that cause malaria, especially Plasmodium species. Malaria causes the death of about 580,000 annually, the majority of them being minors (Venkatesan, 2024)

Malaria is endemic in more than 85 nations accounting for 40% of the global population (Kawaguchi et al., 2022; World Health Organization (WHO), 2022). In areas of continuous transmission of malaria, children <5 years of age and pregnant women experience the most morbidity and mortality from the disease(Phillips et al., 2017). The World Health Organisation (2023) has classified Sub-Saharan Africa as the world's most malaria-endemic zone. Over 90% of the 580,000 malaria deaths worldwide were in Sub-Saharan Africa, of which over 260,000 were in paediatrics(Sarfo, Amoadu, Gyan, et al., 2023; Sarfo, Amoadu, Kordorwu, et al., 2023). This means that one child will pass away every two minutes.

No medication is universally effective in treating malaria and despite numerous ongoing efforts to create a vaccine, there is no single vaccine that is fully effective ((Garcia, 2010; Okoro et al., 2020; Venkatesan, 2024). Prevention of malaria is also difficult due to the need to control mosquitoes and conduct awareness (Diema Konlan et al., 2019). The burden of malaria infection among school-age children in sub-Saharan Africa (ages 5 to 15) is a major source of *Plasmodium falciparum* transmission from humans to mosquitoes (Cohee et al., 2020).

Plasmodium species such as *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowles* are the common plasmodium species that infect humans (Phillips et al., 2017). The most prevalent parasite, *Plasmodium falciparum*, is more likely to cause fatal malaria. According to the World Malaria Report (2022), *Plasmodium ovale* is primarily restricted to central West Africa and a few South

Pacific islands, *Plasmodium falciparum* is typically limited to the tropics and *Plasmodium malariae* is occasionally found around the world.

During the rainy season and in warmer climates, malaria is more frequently transmitted (Mhalu, 2005). The life cycle of *Plasmodium falciparum* is shared by humans and female anopheline mosquitoes. The mosquito injects sporozoites as it feeds on human blood. During infection, the sporozoites first infect liver cells, where they proliferate throughout a seven-day asymptomatic replication cycle. Hepatocytes release invasive merozoites, which infect red blood cells and cause the parasites to repeatedly proliferate, replicate, emigrate, and invade. Fever, headaches, and exhaustion are some of the symptoms that occur during this phase of erythrocyte infection, which is known as the symptomatic illness (Shahbodaghi & Rathjen, 2022; Zdrodowska et al., 2006).

Epidemiology of paediatric malaria in Malawi

One of the causes of illness and death, particularly in Sub-Saharan African nations, is malaria. Paediatrics and pregnant women are the most vulnerable populations when it comes to malaria (Semakula et al., 2023). Malawi is found in the tropics and among the countries in sub-Saharan Africa where malaria is endemic. In 2023, the World Health Organisation Malaria Report showed that Malawi holds 1.8% of the total malaria burden and 1.2% of the total deaths in paediatrics. In a study by (Gaston et al., 2021; Gaston & Ramroop, 2020), the prevalence of malaria was estimated to be 37.2% of all the children who were tested using mRDTs. Malaria accounted for 20% of all deaths in the paediatrics. This indicates that malaria is a serious health concern in the nation and that additional studies employing diverse techniques are

required to pinpoint the risk factors linked to the illness and develop novel diagnostic approaches, particularly for children under five.

Different other studies have indicated the prevalence and incidence of malaria in Malawi. Studies looking at determinants of malaria infection established that, 35.4% of paediatrics had malaria at hospital visitation. The results suggested factors such as gender-based violence, education level and age were associated with the level of risk of being infected with malaria. Most Malawian mothers have less to no secondary school education and thus the risk of malaria is higher(Chilanga et al., 2020). This increase in risk can be attributed to a lack of knowledge of malaria prevention methods.

Epidemiology of non-malarial fever in Malawi

Data retrieved from Malawi has demonstrated that non-malarial illness prevalence in Malawi was at 72% in children < 5 years. Among febrile patients with negative mRDT results, respiratory infections (46%) were the most common clinical diagnosis, followed by sepsis (29%), gastroenteritis (13%), musculoskeletal discomfort (9%) and malaria (5%) (Kapito-Tembo et al., 2020). Many other studies have demonstrated that at least 50% of fever in paediatrics is non-malarial fever ((Baltzell et al., 2019). Studies have shown that most non-malarial fevers are mainly caused by bacterial infections and less commonly parasitic and virial aetilogy (Wainaina et al., 2022). This suggests that in areas where there is a lack of diagnostic tests, malaria over-diagnosis can occur due to overlapping symptoms.

Clinical presentation of malaria, viral and bacterial infections

Manifestations of infectious diseases may be local such as in abscess or systemic in which fever is most common (El-Radhi, 2019). Severe systemic infections may have life-threatening conditions such as septic shock and sepsis. Most manifestations resolve with successful treatment of the underlying infection. The clinical symptoms and progression of the disease depend on age, inoculation dose and virulence of the infecting pathogen (van Seventer & Hochberg, 2017). Most viral and bacterial systemic infections lead to sepsis which may present as fever and other organ symptoms (El-Radhi, 2019).

Sepsis is a condition characterised by a dysregulated response to infection and potentially leading to a fatal organ failure. The immune system is triggered when a pathogen enters the body, causing the production of several pro-inflammatory cytokines and acute-phase proteins. Sepsis results from this reaction if it is not controlled (Henriquez-Camacho & Losa, 2014; Minderhoud et al., 2020; van Engelen et al., 2018). Different gram-positive and negative bacterial species are common causes of sepsis in individuals. The indications and symptoms in a clinical context include fever, hypotension, oliguria and disorientation (Baltzell et al., 2019). The cross-presentation of clinical symptoms in different infectious diseases leads to diagnostic turmoil.

Malaria mainly presents as an uncomplicated febrile illness associated with fever, vomiting, general body pains and headache(Frimpong et al., 2022; Obeng-Aboagye et al., 2023). In many cases, malaria resolves within a short time but in 1% to 2 % of cases, malaria advances to a serious potentially fatal illness (Angchaisuksiri, 2014). Immune state, age and exposure may all have an impact on this progression. Malaria symptoms include, but are not limited to, fever, headaches, and overall body weakness (Wassmer et al., 2015).

The challenge of finding the causative agent of fever in malaria-endemic regions

The majority of paediatric patients in resource-limited countries present with acute fever. This is often attributed to malaria and treatment is given without any laboratory evidence ((Penda et al., 2023). In developing countries like Malawi, laboratory infrastructure and personnel are inadequate making testing more challenging (Chilanga et al., 2020).

In endemic areas, mainly the tropics, Malaria often co-exist with other infectious diseases such as bacterial and viral infections. The co-existence leads to misdiagnosis, poor prognosis and poor differentiation between malarial fever and other infectious diseases (Long, 2016). The challenge is escalated due to the overlapping of clinical signs and symptoms between malaria and non-malaria fever. The available RDTs, culture and microscopy have limitations and in certain circumstances cannot identify the source of fever (Baltzell et al., 2019). Finding the fever source becomes a challenge, especially in resource-limited areas where technology and personnel are scarce.

A lot of studies have been done to find biomarkers in many viral diseases and inflammation because of the shortcomings of existing diagnostic procedures, which may have low sensitivity and extended turnaround times. In this situation, a biomarker could quickly and accurately differentiate non-infectious causes of systemic inflammatory disease from bacterial, viral and parasite infections (Califf, 2018). By doing this, a biomarker might be employed to help with treatment planning and decision-making by enabling the early detection of sepsis, viral infections and parasite infections.

Diagnostic approaches to bacterial, viral and parasitic infections

Both adults and children contract infections regularly. New diagnostic techniques have allowed us to learn more about pathophysiology and epidemiology of infectious diseases. Recently, emerging infections have surfaced, prompting health authorities to issue alerts. In addition, novel anti-infective therapies have been identified; however, it is critical to understand when to employ them to prevent drug abuse and misuse, which may result in adverse effects such as antimicrobial resistance (Chiappini et al., 2009; Průcha et al., 2018; Yang & Rothman, 2004).

Malaria diagnosis

Different geographic regions are home to different Plasmodium species that cause malaria. Many areas have extensive infection with rising medication resistance, and the illness share clinical similarities with other infectious diseases (Mandala et al., 2022). The control of malaria involves strategies for controlling mosquito populations and also quick methods of diagnosing and treating malaria cases (Hemingway, 2015).

Malaria diagnosis in Malawi relies on multiple techniques to identify the presence of parasites in blood (Baltzell et al., 2019). Although rapid diagnostic tests (RDTs) have quickly taken the place of primary diagnostic tests in many endemic areas, microscopy remains the oldest method and the gold standard (Sarfo, Amoadu, Kordorwu, et al., 2023). For malaria laboratory diagnosis, using a light microscope, blood smear slides are examined and parasite densities are noted (Bisoffi et al., 2012). Microscopy has limitations such as low sensitivity, it's time-consuming and there is a need for a skilled and

experienced technician to view the slides (Mahende et al., 2016; Varo et al., 2020).

There is a need for more precise diagnostic techniques because many slides that test positive for malaria are classified as negative because of low parasite densities (Cardona-Arias et al., 2020). Investigations have discovered notable false positive and false negative results when Plasmodium parasites are routinely rechecked using microscopy on slides (Elven et al., 2020). Additionally, there was an over reporting of malaria in Malawi and a reported lower grade in parasite species identification on panel testing ((Gidey et al., 2021; Nega et al., 2020). The creation of mRDTs was prompted by this difficulty.

On the other hand, mRDTs are mostly used in endemic areas. They are designed to detect specific malaria antigens or enzymes. The current mRDTs use plasmodial lactate dehydrogenase (pLDH), plasmodial aldolase and *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2). More than 90% of malaria RDTs now in use are tests that target HRP2. However, these tests' specificities, sensitivities, false positive and false negative rates, and temperature tolerances vary widely, highlighting the problems and obstacles in the performance (Mouatcho & Dean Goldring, 2013; Requena et al., 2015). The rapid tests are also specific to a species of plasmodium and also the concentration of the target protein, this makes it difficult to identify other species in areas where the most prevalent is *Plasmodium falciparum*. The targets are also prone to mutation and hence false negatives (Feleke et al., 2021; Jimenez et al., 2017). This complicates the whole process of malaria diagnosis and cements the need for new markers for testing.

Additionally, the syndromic approach is the most common method of malaria diagnosis in resource-scarce settings. This method is based on symptoms like fever, fatigue and headache to diagnose malaria (Varo et al., 2020). It is more unreliable due to overlap with other febrile illnesses such as bacterial infections and the risk of misdiagnosis is high. In different studies across sub-Saharan Africa, it was indicated that there is a challenge in differentiating malarial fever from non-malarial fever based on this method (Mahende et al., 2016). Owing to this, a new simple method using biomarkers that can work in rural settings can alleviate the challenge.

Bacterial infection identification methods

Bacterial infections are commonly diagnosed using traditional culture-based methods, wherein bacterial pathogens are isolated and identified by the inoculation of clinical specimens on a specific medium (Herlihy et al., 2016). However, these methods are time-consuming and may not provide rapid results necessary for timely patient management. For the identification of bacteria, ELISA and molecular methods such nucleic acid amplification tests and polymerase chain reaction provide quicker and more accurate options (Průcha et al., 2018; van Seventer & Hochberg, 2017). These molecular methods have revolutionized bacterial diagnosis by providing rapid and accurate results, enabling prompt initiation of targeted antimicrobial therapy. The challenge with molecular methods is that they are expensive and impractical in resource-scarce situations (Azad & Patel, 2024) . Different studies have investigated the expression of markers such as CRP, procalcitonin and lactate in bacterial and viral diagnosis with promising results (Mathur & Behera, 2019; Sridharan &

Chamberlain, 2013; Xiao et al., 2017). Even though there have been extensive studies, many of these markers never made it to the final stage of utility.

Viral infection identification methods

Unlike bacterial infections, viral infections pose unique challenges in diagnosis due to the lack of reliable culture-based methods for most viruses. Instead, viral identification relies heavily on serological assays, antigen detection tests and molecular techniques (S. Wang et al., 2024). Viral-specific antibodies or antigens are found in patient serum by serological assays, such as RDTs, neutralisation tests and enzyme-linked immunosorbent assays (ELISA), which indicate viral exposure (Li et al., 2021). A good example of this is the recent testing of COVID-19 antigen-based assays using rapid tests. Antigen detection tests, including rapid antigen tests and immunofluorescence assays, identify viral antigens in clinical specimens directly, thereby providing rapid diagnosis of acute viral infections (Binnicker, 2015). Furthermore, high sensitivity and specificity viral nucleic acid identification and quantification are made possible by molecular techniques such as real-time PCR and reverse transcription PCR (RT-PCR) (Yang & Rothman, 2004). These molecular assays play a critical role in diagnosing viral infections, particularly in outbreak investigations and monitoring disease trends.

Advancements and future directions in diagnosis dynamics

Advancements in technology continue to enhance bacterial and viral identification methods, paving the way for improved diagnostic accuracy and efficiency. Next-generation sequencing (NGS) technologies offer methods for characterising and identifying microbes that enable the simultaneous

identification of viral and bacterial pathogens in clinical specimens (van Seventer & Hochberg, 2017; Yang & Rothman, 2004). Furthermore, the advancement of multiplex assays and point-of-care testing (POCT) equipment allows for the quick and simultaneous identification of several infections, which speeds up the diagnostic and treatment selection process (Manca et al., 2017; Tsalik et al., 2017). The amalgamation of artificial intelligence and machine learning algorithms augments the precision of diagnosis by scrutinising intricate datasets and pinpointing distinct biomarkers linked to both bacterial and viral ailments. These advancements hold promise for transforming infectious disease diagnosis and surveillance, ultimately improving patient outcomes and public health response.

Immune responses to infections

The immune system functions in emergence, persistence and elimination of numerous disorders that threaten the human health. When a pathogen—such as bacteria, virus, or protozoa— enters the body, the immune system gets activated. To help fight the infection, it starts producing pro-inflammatory cytokines like IL-6 as well as acute-phase proteins like CRP. This initial line of defence is provided through the innate immune system (McComb et al., 2019). The infectious agent enters the tissue when the physical and chemical barriers break down. Through the action of phagocytic cells, natural killer cells, blood proteins, various inflammatory mediators and cytokines, the tissue's innate response will fight off the infection continuously (Netea et al., 2020; Nkansah et al., 2024). The body also uses the adaptive mechanism through the production of antibodies and cytotoxic killer cells. During these

processes, a lot of proteins are produced as signalling molecules and some enhance the immune reaction (Netea et al., 2020; Nkansah et al., 2024; Ong'echa et al., 2011). All these proteins when measured in the serum or cell surface can act as biomarkers for diagnosis or disease prognosis.

Biomarkers in fever

The body reacts to infections and other stressors with fever, which is an intense yet nonspecific reaction. It is caused by immune cells releasing cytokines, which raise body temperature through the action of the brain prostanoid. Cytokines and other proteins can work as markers of disease presence or absence (Póvoa et al., 2020). In this context, biomarkers could be indicators of infection, dysregulated host response, therapeutic response, or help medical professionals anticipate patient danger. In the previous several decades, more than 250 biomarkers have been discovered and examined. Data that have been published support the use of biomarkers for clinical diagnosis, pathogen identification, and antibiotic therapy optimisation (Balerdi-Sarasola et al., 2023).

Biomarkers are biological characteristics that can be assessed objectively and utilised as an indicator of a drug's activity or as an indicator of a pathological or physiological process (Bodaghi et al., 2023; Califf, 2018). Compared to directly measuring the final clinical outcome, using clinical biomarkers is simpler, less expensive and typically involves measuring the biomarkers over a shorter period. Because of this, biomarkers have applications in the diagnosis, prognosis and surveillance of infectious diseases.

Numerous research has examined the predictive power of inflammatory indicators and the COVID-19 pandemic has heightened interest in inflammatory biomarkers for viral infections (Póvoa et al., 2020; Póvoa & Coelho, 2021). The expression of biomarkers for malaria and non-malarial fever, C-reactive protein, ICAM-1, Interleukin -6, Interleukin -1 beta, Interleukin -10, ANG-2 and vWF-1 have been examined in this work to differentiate fever etiology in pediatrics presenting with fever.

C-reactive protein (CRP)

CRP is a protein produced by the liver. CRP acts as an acute-phase reactant protein and a marker of an inflammatory process (Bhattacharya & Munshi, 2023; Pathak & Agrawal, 2019). The levels of CRP have demonstrated an increase in response to inflammation due to many infectious diseases ((Noh et al., 2021), CRP exhibits both pro- and anti-inflammatory characteristics (Rizo-Téllez et al., 2023). It also contributes to the identification and removal of damaged cells and foreign infections. This is accomplished by adhering to the surfaces of injured cells, which include phospholipids, chromatin, histones and fibronectin. It can hasten the removal of cellular debris, apoptotic cells and foreign pathogens by activating the complement system and phagocytic cells via Fc receptors (Bhattacharya & Munshi, 2023; Pathak & Agrawal, 2019)

Different studies have looked at CRP and how it is expressed in different diseases. Its relevance is mainly in the detection of fever caused by bacterial infections such as *Streptococcus pneumoniae* and Salmonellosis. Patients presenting with bacterial infections have a remarkable increase in CRP levels than those having either malaria or viral infections (Kilpatrick et al., 2024). Another study by Bhardwaj *et al*. (2019), agreed with Kilpatrick et al. (2024)

that elevated CRP levels are in bacterial infections than malarial infections and this can help in diagnosis. This therefore makes CRP a good marker for bacterial infection. Reaching this far, CRP biomarkers have been developed into rapid tests and are being used in peripheral health settings to identify bacterial infections.

However, a study in Cameroonian pediatrics found that the role of CRP in excluding malarial fever from other sources of fever is not significant (Y. Ngwengi et al., 2023). According to Ngwengi et al. (2023), there is no discernible difference between the mean CRP levels of bacterial infection and malaria infection. Furthermore, it was shown that the means of CRP were considerably higher in cases of malaria or bacterial infection compared to cases of viral infection. This finding implies that CRP is not a valid biomarker for differentiating bacterial from malarial infections and thus calls for further investigation.

In Malawian pediatrics, CRP has been studied in the context of serious bacterial infection. It was shown that on admission CRP levels correctly predicted the bacterial infection, particularly pneumococcal bacteremia (Connon et al., 2021) but did not perform better in differentiation of malarial from bacterial fever. Therefore, having a platform where CRP is measured in both malaria, bacterial and viral fever can assist in coming up with a profile of cut of values to be used in diagnosis.

Intercellular adhesion molecule 1 (ICAM-1)

The transmembrane glycoprotein ICAM-1, commonly referred to as CD54, is essential for leukocyte trans-endothelial migration. Elevated levels of ICAM-1 indicate endothelial injury, which can result from infections or other

inflammatory conditions (Bui et al., 2020; Zhai et al., 2021). During systemic inflammatory responses, ICAM-1 expression increases due to dysregulated inflammatory cytokines. Tissue injury can result from inflammation due to the increased expression of adhesion molecules like ICAM-1. The ICAM-1 can be measured as soluble ICAM-1 (sICAM-1) in serum. ICAM-1 enables the infiltration of immune cells to the site of inflammation (Obeng-Aboagye et al., 2023), causing mediators like chemokines and cytokines to be secreted.

In non-malarial infections, a remarkable increase of ICAM-1 has been evident in viral infections such as COVID-19 and HIV (Yu et al., 2020) where ICAM-1 facilitates inflammatory cell recruitment and activation. Beyond its role in inflammation, ICAM-1 also serves as a receptor for viral entry, notably in Rhinovirus infections of lung epithelial cells (Shukla et al., 2022). ICAM-1 elevation is not exclusive to viral infections. Bacterial infections like Bacterial Vaginosis and *Mycobacteria Leprae*, as well as intestinal bacterial infections, have demonstrated higher levels (Alcaide et al., 2017; Thurman et al., 2015)

In malaria, ICAM-1 level correlates with endothelial activation triggered by the sequestration of infected erythrocytes (Suurbaar et al., 2022). In the brain, *Plasmodium falciparum* can bind to receptors such as ICAM-1 and endothelial protein C receptor (EPCR) to cytoadhere to the endothelium (Rowe *et al*., 2009) leading to endothelial activation. In severe episodes of malaria, such as cerebral malaria in children from Malawi, elevated levels of ICAM-1 have been reported (Conroy et al., 2010). Furthermore, compared to uninfected controls, children with asymptomatic parasitaemia exhibit noticeably higher ICAM-1 levels in Ghana (Frimpong et al., 2020). This is supported by (Zeukeng et al., 2014) who noted elevated ICAM-1 plasma levels in Cameroonian malaria

patients. This implies that ICAM-1 can act as reliable indicator of the severity of malaria.

Angiopoietin-2 (Ang-2)

Angiopoetin-2 is an indicator of endothelial activity. A class of growth factors called angiopoietins is necessary for the division of existing blood vessels into new ones (Conroy et al., 2010). In this family, angiopoietin-1 (Ang1) and angiopoietin-2 (Ang2) are the main members. To maintain vessel integrity and enhance cell survival, Ang-1 stimulates blood vessel development and stability via binding to the Tie2 receptor on endothelial cells (Ma et al., 2020). Conversely, Ang-2 operates as an agonist or antagonist of Ang1-Tie2 signalling depending on the circumstances, often promoting vascular remodelling and destabilisation (Conroy et al., 2010). This process is critical for the development of tumours and inflammation. Maintaining appropriate vascular homeostasis and responding to both physiological and pathological stimuli require Ang1 and Ang2 activity balance (Akwii et al., 2019).

Limited data is available on the utility of Ang-2 in differentiating malarial fever and non-malarial fever. Many studies have investigated Ang-2 in either malaria or bacterial infection but not in the same cohort. Numerous investigations have shown that endothelial activation may be measured quantitatively using the biomarker angiopoietin 2, which is present in infectious illnesses such as bacterial pneumonia and COVID-19 (Alay & Laloglu, 2021). Studies conducted to investigate endothelial activation (Ma et al., 2020; L. Zhang et al., 2022) found a correlation between the severity and prognosis of paediatric pneumonia and high admission levels of Ang-2. Ang-2 thus is suggested to be a reliable indicator of the severity of the infectious condition.

In terms of malarial fever, elevated levels of Ang-2 levels between uncomplicated and severe malaria cases in contrast to the control group were noted in infected participants (Oluboyo et al., 2020). In an investigation of patients from two different populations—Thai adults and Ugandan children—who had uncomplicated malaria and were infected with *Plasmodium falciparum*, Ang-2 levels were found to be considerably higher in both populations with malaria than in controls (Lovegrove et al., 2009). Similarly, Malawian children suffering from cerebral malaria had elevated levels of Ang-2 in comparison to those with uncomplicated malaria and control groups (Conroy et al., 2010). These findings imply that Ang-2 can function more effectively as a measure of severity of malaria infection.

In a quest to find malaria biomarkers, (Ma et al., 2020) created a model with the highest sensitivity and specificity that combined Ang-2 and CRP. Combining Ang-2 and CRP could be a useful method for spotting severe malaria early on. Using a fast predictive test that incorporates the previously described indicators could improve the management of malaria and potentially lower the number of hospitalisations and complications among affected patients.

von Willebrand factor -1(vWF-1)

According to (Ma et al., 2020), vWF-1 is a sizable glycoprotein that is necessary for hemostasis, the process that ends bleeding. The main function of vWF-1 at the sites of vascular damage is to facilitate platelet adhesion and aggregation (Stockschlaeder et al., 2014). In injured blood arteries and platelets, vWF-1 attaches to exposed collagen to promote the creation of a platelet plug. vWF-1 also acts as a carrier protein for factor VIII, preventing it from degrading

quickly and prolonging its half-life in circulation (Lenting et al., 2015; Seidizadeh et al., 2024).

Malaria and other infectious diseases cause vascular injury and hence it can lead to vWF-1 production. Infectious diseases disrupt blood vessel function through endothelial cell activation. According to studies, these alterations may be observable and act as biomarkers for infectious disease detection. Patients with malaria often have blood coagulation activation, which raises vWF-1 (Lenting et al., 2015; Ma et al., 2020; J. W. Wang et al., 2012). Disseminated intravascular coagulation (DIC) is clinically linked to a highly serious illness with a high death rate. In individuals with *Plasmodium falciparum* infection, elevated plasma levels of von Willebrand factor-1 (vWF-1) and endothelial microparticles have been observed (. It has been shown that aberrant circulating ultra-large vWF-1 multimers and acute endothelial cell (EC) activation are linked to severe *Plasmodium falciparum* infection (Angchaisuksiri, 2014). However, a study by (Masse & Hantson, 2014) showed that a malaria patient's serum had signs of decreased von Willebrand factor-1 (vWF-1) activity. This implies that more investigation is required to comprehend how vWF-1 manifests in malaria instances.

In another study, S*taphylococcus aureus* infection was connected to elevated von Willebrand factor-1 levels (vWF-1). It is thought that vWF-1 binds to the bacteria cell wall and influences its growth and also activates the endothelial cells ((Steinert et al., 2020). Another study in *Streptococcus pneumoniae* demonstrated that infection with *Streptococcus pneumoniae* causes the body to release vWF-1 hence its increase (Jagau et al., 2019). It is therefore

important to study vWF-1 in malarial and non-malarial fever and observe the trends.

Cytokines definitions and classifications

Inflammatory and infectious disorders trigger the immune system to mount a suitable defence that involves cytokine production. Cytokines are small proteins that are crucial in cell signalling (Qiao et al., 2019). Cytokines are released by cells and affect the behaviour of self or other cells. Pathological and physiological processes such as inflammation, immunology and hemopoiesis are influenced by cytokines (Silva et al., 2019). They can act in an autocrine, paracrine or endocrine manner. A range of cells, including immune cells like T lymphocytes and macrophages as well as non-immune cells like fibroblasts and epithelial cells, produce cytokines (Tang et al., 2022).

Cytokines are categorized into several types based on their function. Pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 stimulate immunological responses and inflammation. To stop tissue damage, anti-inflammatory cytokines like TGF-β and IL-10 reduce inflammation(de Oliveira et al., 2011). Growth and differentiation cytokines like IL-2 and GM-CSF stimulate cell proliferation and survival and chemokines, including IL-8 and MCP-1 direct the migration of immune cells to sites of infection (Jarczak & Nierhaus, 2022). Interferons, such as IFN-α, IFN-β, and IFN-γ, are cytokines that influence the immune system and provide protection against infections (Kaneko et al., 2019).

In reaction to several stimuli, white blood cells and other cells secrete regulating proteins called interleukins. The source of this stimulus may be an infection or a normal bodily process like growth and balance (Jarczak &

Nierhaus, 2022). Interleukins are produced by cells including lymphocytes, monocytes and macrophages so they can communicate with other cells. Interleukins have several roles, including triggering the production of antibodies by B cells and drawing immune cells, including lymphocytes and macrophages, to infection sites (Li et al., 2021; Mandala et al., 2022). All these cytokines work in connection to mount an immune response against infections such as malaria and also offer homeostasis.

Interleukin 6 (IL-6)

In the innate immune response, interleukin 6 (IL-6) is an essential cytokine. IL-6 induces the production of CRP and functions in the clusters of differentiation of cells (Rose-John, 2018). Also, IL-6 triggers the activation of haptoglobin, fibrinogen, serum amyloid A, CRP, and α1-antichymotrypsin during inflammation(Majidpoor & Mortezaee, 2022). This activation is important in the immune against infections because it amplifies inflammation, promotes blood clotting and prevents oxidative damage (McElvaney et al., 2021). Conversely, IL-6 decreases transferrin, albumin, and fibronectin synthesis (Tanaka et al., 2014). This is done to conserve energy and also reduce iron which may aid in pathogen growth. Levels of IL-6 have been shown to increase in both malarial fever and non-malarial fever.

IL-6 has been linked to severe malaria cases, even if it's still uncertain if this connection is causative or if modifying IL-6 levels could affect outcomes in severe malaria (Hamilton & John, 2013). According to (Wilairatana et al., 2022), large levels of interleukin-6 are produced when a person is infected with malaria parasites. This release is linked to malaria's immunopathogenesis. Patients with severe malaria exhibit mean IL-6 levels that are greater than those

of controls and non-severe malaria patients. Moreover, the mean IL-6 levels of patients with uncomplicated malaria are higher than those of the controls. Similarly, Mandala et al., (2022), discovered that children from Malawi who had malaria had elevated levels of IL-6. Patients suffering from cerebral malaria exhibited the highest levels of IL-6 among the three forms of malaria, indicating that IL-6 may be a useful indicator of the severity of malaria.

The expression of IL-6 is not only limited to malaria infection. In a study by Qiao & Fu. (2020), children infected with adenovirus and coronavirus expressed higher levels of IL-6. In another study by (García-Hernández et al., 2016), Interleukin-6 demonstrated superior diagnostic accuracy for bacterial meningitis (AUC = 0.937, 95% CI: 0.895-0.978) from aseptic meningitis compared to traditional biomarkers. This is also supported by a comparison of biomarkers in malaria infection and bacteremia (Post et al., 2021) discovered that IL-6 levels in bacteremia cases were considerably higher than in malaria and health controls Burkina Faso Plasmodium Falciparum infection. Furthermore, previous research has demonstrated that in feverish newborns, interleukin-6 is a sensitive and specific marker for the presence of a severe bacterial infection(Pratt & Attia, 2007; Vujevic et al., 2017).This therefore needs further investigations to ascertain the involvement of IL-6 in fever caused by different infectious diseases including malaria.

Interleukin 1 beta (IL-1β)

One well-studied pro-inflammatory protein in the interleukin-1 (IL-1) family is IL-1β. IL-1β is a key mediator of inflammation that causes fever and immunological activation by binding to IL-1 receptor 1 (Kaneko et al., 2019). IL-1β triggers inflammatory signalling pathways that induce cytokine

production, immune cell activation and tissue responses crucial for defence and repair during infections and inflammatory conditions (Yazdi & Ghoreschi, 2016a).

During malaria infection, particularly with *Plasmodium falciparum*, erythrocytes become infected. This sets off the host's innate immune response, which causes monocytes and macrophages to produce pro-inflammatory cytokines, such as IL-1β. Fever, chills and other typical malaria symptoms are caused by IL-1β, which is a key player in the inflammatory cascade (Mota & Madden, 2022). Studies have shown that plasma concentrations of IL-1β are considerably greater in cases of severe malaria (Acero et al., 2022; Mahittikorn et al., 2022), and malaria coinfections than in uncomplicated malaria and controls (Dinarello, 2011; Kotepui et al., 2024; Yazdi & Ghoreschi, 2016b). This shows that IL-1β can be used to define malaria severity.

Additionally, studies have revealed that patients with bacterial sepsis have higher levels of IL-1β than patients with malarial illnesses (Baltzell et al., 2019). This difference likely arises from the distinct immune response patterns elicited by malaria parasites and bacterial infection. Furthermore, in bacterial infections, early reactions to *Staphylococcus aureus* bacteriemia are exhibited by IL-1β, which is also linked to the length and fatality of the illness (Dinarello, 2011). Moreover, fever attacks in patients with mediterranean viral fever have been found to significantly elevate serum concentrations of pro-inflammatory cytokines IL-1β when compared to control (Çaldıran et al., 2021). In a Vietnamese study of dengue fever, the clinical outcome of dengue infection was examined in connection to the levels of IL-1β in patients. The findings demonstrated that dengue patients' IL-1β levels were considerably higher than

those of health controls (Tuyen et al., 2020). This cross increase of IL-1β among malarial and non-malarial fever requires further investigation to ascertain its role in the inflammation process.

Interleukin 10 (IL-10)

One important anti-inflammatory cytokine that is essential for controlling immune responses and preserving immunological homeostasis is interleukin-10. T cells, B cells, macrophages, dendritic cells and regulatory T cells are among the immune cells that produce IL-10 (Edwards, Ng, de Labastida Rivera, et al., 2023). It works by inhibiting the synthesis of pro-inflammatory cytokines, including interleukin-1, interleukin-6, interleukin-12 and tumour necrosis factor-alpha (Saraiva et al., 2020). Nevertheless, overproduction of IL-10 may impair the body's ability to mount an efficient defence against diseases, such as malaria, even though it is necessary for immunological tolerance maintenance and the prevention of excessive inflammation (Acero et al., 2022; Lyke et al., 2004).

Research on IL-10 has highlighted its dual role in immunity, acting as a double-edged sword by both suppressing inflammatory responses and potentially impeding pathogen clearance (Saraiva et al., 2020). Understanding the regulation of production of IL-10 and its effects on immune function is crucial for developing therapeutic strategies for various diseases, including autoimmune disorders, inflammatory conditions and infectious diseases (Kumar et al., 2019).

IL-10 levels in malaria have shown different outcomes in line with diagnosis. While some studies show a correlation with severity, others show an inverse relationship. As part of the immune system's reaction to the malaria

parasite, IL-10 production is frequently increased in cases of malaria fever (Lyke et al., 2004). Studies have shown that IL-10 can help regulate inflammation and prevent tissue damage during malaria infection (Edwards, Ng, De Labastida Rivera, et al., 2023). As observed in severe malaria syndromes, elevated IL-10 levels in malaria fever may be linked to the severity and course of the disease. Monitoring IL-10 levels in malaria fever patients could provide insights into the immune response and potential complication. On the other hand, a negative association between the expression of IL-10 and parasitemia and the severity of the disease was found in a study by Mahanta et al. (2015), indicating that IL-10 is the primary cytokine involved in tipping the scales in favour of an inflammatory response. However, because these indicators lack specificity, it is recommended to combine several biomarkers implicated in several malaria pathophysiology pathways.

In terms of non-malarial fever, studies have found that 1L-10 can aid in the early diagnosis of bloodstream infections and hence help in quick diagnosis. Significant variations were noted between the IL-10 levels of individuals with bloodstream infections caused by bacteria and those in the control group (Kumar et al., 2019). Similar to this, L. Zhang et al. (2022),showed that IL-10 in addition to other cytokines can be helpful in the detection and differentiating of bacteraemia and lung bacterial infection.

Studies combining malarial and bacterial or viral infections have demonstrated an increase in IL-10 in malaria patients than in bacterial infections. In Malawian paediatrics, IL-10 was found to be significantly higher in malarial sepsis than in non-malarial sepsis (Baltzell et al., 2019). The increase

in IL-10 in malarial fever shows that there is a stronger anti-inflammatory response in malarial fever.

Chapter summary

From this literature review, it has been demonstrated that IL-10, IL-1, IL-1β, ICAM-1, vWF-1, Ang-2 and CRP are both expressed in infectious diseases such as malaria and non-malarial fever. IL-10 acts as a good marker of malarial infection and severity. IL-6 and IL-1 beta have shown a potential to identify non-malarial fever.

CHAPTER THREE

RESEARCH METHODS

Introduction

This study aimed at finding serum biomarkers distinguishing malaria from non-malaria fever in paediatric patients in Blantyre District in Malawi. This chapter discusses the research design, study place, sample collection and analysis methods. The chapter also discusses data interpretation strategies that were used and ethical clearance.

Research design

To find serum biomarkers distinguishing malaria from non-malaria fever in pediatric patients in Blantyre District, a retrospective cross-sectional study was conducted. 90 Serum samples from pediatrics were taken from archives of a previous study titled "Understanding the Immunological Basis of Concurrent *Plasmodium falciparum* Malaria and Invasive Bacterial Diseases in Malawian Children". All serum samples were collected from pediatrics aged between 6–60 months who presented with fever at the time of visiting Queen Elizabeth Central hospital or any of the satellite clinics in Blantyre, Malawi. Amongst the 90 samples, 53 were males and 47 were females. The children included in the study were recruited from a variety of backgrounds, reflecting the heterogeneity of the population that seeks medical care in the district, which includes both urban and rural children. Queen Elizabeth Central Hospital and surrounding satellite clinics serves a large catchment area and treats a range of infectious diseases that are common in the region. The cross-sectional design

was appropriate for this study because it allowed on-point biomarkers that differentiate the etiology of fever at the point of a hospital visit.

The retrospective nature of this study had a challenge due to a potential bias caused by reliance on data and samples collected for another study, which may limit the ability to control all variables or ensure consistency in sample collection protocols. To address this, a thorough review of the documentation and methods used for data and sample collection from the initial study was done. Documentation of any inconsistencies or variations that could affect the interpretation of results was also conducted.

Study area

The study was carried out in Blantyre which is located in southern Malawi with a population of approximately one million people. Blantyre is anchored by Queen Elizabeth Central Hospital, a regional referral facility, along with numerous satellite clinics. Annually, these healthcare centres receive over 900,000 paediatrics, both locally and through referrals from surrounding areas. Blantyre's tropical climate supports a thriving population of mosquitoes and other vectors that transmit infectious diseases. Despite being a developing nation with high poverty rates, Malawi face challenges in equipping its healthcare system with adequate resources required for precise infectious illness diagnosis and treatment.

Population, sampling procedure and inclusion criteria

Population, sampling methods and inclusion criteria used in the initial study

The initial study collected 156 blood samples from pediatrics who presented with fever in Blantyre, Malawi. The initial study collected blood samples from male and female pediatrics aged between 6–60 months. Children with no fever, malaria, or a history of sickness within the preceding three months served as controls. Convenience sampling was used to identify study participants. At the time of enrolment, three sets of paediatrics were recruited. The fever group consisted of those with an axillary temperature of > 37.5°C. This was further divided into malarial and non-malarial fever based on MRDT results. Children having HIV infection, chronic illness, hypoglycaemia (serum glucose < 45 mg/dL), severe anaemia, and abnormal Blantyre coma score were excluded. Study field workers gathered healthy controls from the immunisation clinic who had no fever or malaria. Each participant underwent a clinical assessment conducted by a skilled research nurse. Venous blood samples (3 mL) were drawn from each participant at the time of recruitment and analysed within 4 hours. Blood samples were processed into whole blood, plasma, serum and dried blood spots for specific analysis (Nyirenda et al., 2015). At the point of enrolment, all blood samples were subjected to a Malaria rapid diagnostic test using the SD Bio line Malaria Antigen Pf RDT for *Plasmodium falciparum.* This separated participants into malaria fever and non-malaria fever and health controls. Thick blood smear stained with field stains A and B was used to confirm mRDT results. Two qualified, impartial and non-blinded microscopists read and validated the smear. A thin smear stained with Giemsa was used to

identify the species. A Beckman Coulter analyzer was used to provide a full blood count. A thick smear was used to count the number of parasites against the white blood cells (WBCs) (Nyirenda et al., 2015). Written informed consent was obtained from the guardians of all clients. The Malawi College of Medicine Research Ethics Committee granted ethical permission for the study (protocol number P.08/15/1785). All the remaining serum samples were archived at −70°C for storage and further analysis.

Population, sampling methods and inclusion criteria used in the current study

For the current study, 90 serum samples were pulled from the 156 serum samples that were archived at −70°C. This study selected paediatrics with available serum from the pool of paediatrics who presented with fever in the previous study. Fever was defined as a temperature of > 37.5°C. Malaria infection was determined as per the previous study results. Children with fever and mRDT positive were classified as having a malarial fever; children with fever and mRDT negative result were classified as having a non-malarial fever; controls included children without fever and mRDT negative. Controls were from paediatrics with no fever or malaria and no medical conditions in the last 3 months. The serum samples were grouped into malarial fever (n=46), non-malarial fever (n=25) and controls (n=19). Figure 1 shows how serum samples were accorded to groups.

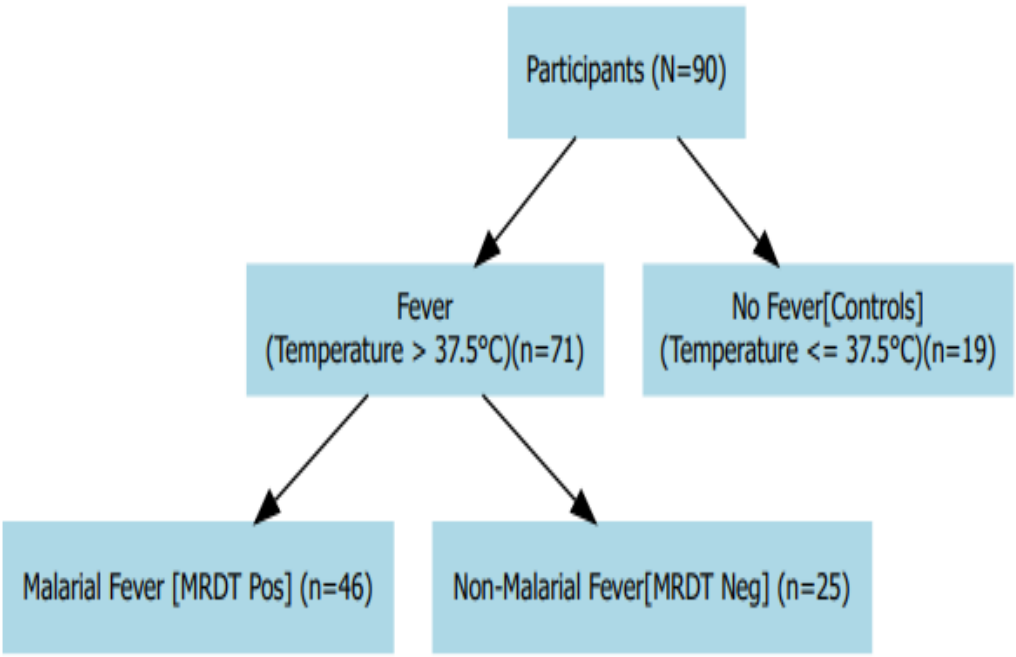

Figure 1: Sample classification criterion used in grouping paediatric samples into malarial, non-malarial fever and controls

Data collection procedures and instruments

Demographics and preliminary Laboratory results

Participants' demographic characteristics such as age and gender; laboratory results including Hgb, platelets, RBC, WBC, parasite count and Glucose; clinical assessment results such as height and weight were collected from the participant enrolment forms that were used in the initial study.

Determination of biomarker concentration using Luminex multiplex bead assay

The current study used Luminex multiplex bead assay (Luminex, Austin, TX) to measure the concentration of biomarkers in serum samples. By following the manufacturer's instructions, Luminex multiplex bead assay was used to measure concentrations of CRP, ICAM-1, IL -6, IL-1β, IL -10, Ang-2

and vWF-1 in paediatric serum samples. To measure biomarker concentration from serum, Multiplex Luminex beads were added to a 96-well plate and washed using an automatic magnetic washer. Each serum sample was loaded to the 96 well-plate in duplicate. Standards having low concentration and high concentration and a blank were added to the same plate. Serum samples were added and incubated in the dark for 2 hours at room temperature. After that, the mixture was washed to remove unbound analytes. A biotinylated detection antibody was added, followed by streptavidin-phycoerythrin (SAPE) incubation. The plate was read using a Luminex machine and biomarker concentrations were calculated by plotting Mean Fluorescence Intensity (MFI) against a standard curve generated from the standard. Data were exported to Excel for further analysis. Luminex assay was chosen because it uses less volume of sample, it is highly sensitive and it can detect multiple analytes at a single point.

To ensure the accuracy and reliability of biomarker measurements, several key quality control steps were implemented during the assay process. All Luminex plates included standards that were supplied by the manufacturer, with concentrations ranging from low concentration to higher concentration. All samples were run in duplicate. Additionally, two blanks containing no sample was included to account for background fluorescence, with the blank's values subtracted from the MFI of the standards and serum samples to correct for background noise. The Luminex machine was calibrated by using calibration beads followed by validation. Furthermore, the Luminex machine is equipped with safe testing function that makes assessment on machine performance, hence ensuring accuracy and reliability.

Data processing and analysis

Firstly, data was entered into a Microsoft Excel file (2019). For all the analysis R statistics software was used. To begin with, specific results for each test were assessed for normality by the Shapiro-Wilk test in the R packages. For Shapiro-Wilk test result of p-value greater than 0.05, indicating normally distributed data, then a two-way ANOVA was applied for further analysis. Conversely, if the Shapiro-Wilk test yielded a p-value less than or equal to 0.05, suggesting non-normal distribution, the significance of variations in the data was assessed using the Kruskal-Wallis method. The Dunnet test (post-hoc) was used to find the p-value between malarial and non-malarial groups, this was done after running either two- way ANOVA or Kruskal Wallis.

To compare the levels of biomarkers, box plots were drawn using R studio. Each box plot represented the median, extreme values, 75^{th} percentile and the 25 percentiles. P values were calculated as discussed above. In order to identify a biomarker that differentiates malaria from non-malarial fever, AUROC was performed in R studio. Biomarkers with a higher ROC were deemed to have a better discriminatory capability. This method allowed for both visual and statistical visualisation.

Finally, machine learning techniques, specifically Support Vector Machine (SVM) was utilized to process the biomarker data and identify patterns that could differentiate between malaria fever and non-malarial fever. This advanced analytical approach allowed for the identification of the most effective biomarkers for distinguishing between different febrile conditions in paediatric patients using precision, recall, sensitivity and F1 scores.

Data visualization techniques, such as box plots, graphs and tables were employed to visually represent the distribution of biomarker levels across different patient groups. These visual representations provided a clear and intuitive way to understand the differences in biomarker expression between malaria fever and non-malarial fevers, aiding in the interpretation of the results.

Chapter summary

The study aimed to identify serum biomarkers to distinguish between malaria and non-malarial fever in paediatric patients in Blantyre, Malawi. A retrospective cross-sectional study design was employed, utilizing baseline data and serum samples from paediatric patients aged 6–60 months presenting with fever at clinical visits. The study analysed biomarker profiles including C-reactive protein, ICAM-1, IL-6, IL -10, IL-1β, vWF-1 and Ang-2 using the Luminex bead assay. By comparing biomarker levels in different patient groups, the study aimed to differentiate between malaria and non-malarial fever. This provides insights into diagnostic accuracy and potential applications in paediatric healthcare. The study's significance lies in addressing gaps in understanding childhood fevers, refining diagnostic strategies and potentially developing new diagnostic tools. Limitations include the focus on paediatric patients which limits generalizability to other age groups.

CHAPTER FOUR

RESULTS AND DISCUSSION

Introduction

This chapter summarises results to identify biomarkers that can differentiate malarial fever from non-malarial fever in paediatric patients presenting with fever. A retrospective study was conducted using 90 serum samples. A thorough analysis of baseline characteristics was undertaken. The data for this analysis was sourced from patient's clinical report forms and laboratory results collected at the point of enrolment in the main study. Serum samples were grouped into malarial fever, non-malarial fever and controls based on the Malaria Rapid Test and temperature. The Luminex multiplex test was employed to quantify the biomarker levels in non-malarial fever, malarial fever and control subjects. All results were analysed using statistical packages in R studio. Analysis such as AUROC, Shapiro, Kruskal Wallis and two-way ANOVA were utilised. For this study, *df*=2 was used in comparing three groups, df=3 was used for comparing 4 groups for either Kruskal Wallis or two-way ANOVA depending on data normality. Data visualisation was done by boxplots, graphs and tables.

Baseline patient results

Temperature

Participants in this study were divided into a fever group and a non-fever group based on a temperature threshold of >37.5°C. Figure 2 illustrates the temperature distributions across malarial fever, non-malarial fever, and control groups. A follow-up group was included to assess whether the clinical

symptoms of malarial fever were short-lived or long-lived at 14 days. The box plot represents the 25th percentile, median, 75th percentile, outliers, while the diamond dot indicates the mean temperature, and small dots represent individual outcomes in the dataset. All temperatures above 37.5°C indicate fever. First, the Shapiro-Wilk test was conducted for the statistical analysis to ensure the normality of the data. For Shapiro-Wilk test result of p-value greater than 0.05, indicating normally distributed data, then a two-way ANOVA was applied for further analysis. Conversely, if the Shapiro-Wilk test yielded a p-value less than or equal to 0.05, suggesting non-normal distribution, the significance of variations in the data was assessed using the Kruskal-Wallis method.

The differences in temperature across the malarial, non-malarial, and control groups were analysed using the Kruskal-Wallis test, yielding a p-value of 0.0089 (test of significance: Kruskal Wallis, Degrees of freedom: 2). The p-value of 0.0089 indicates that with 2 degrees of freedom, the likelihood of the observed differences being due to random chance is very low, leading to conclude that the differences in temperature across the three groups are statistically significant. The analysis revealed that children with malarial fever had higher mean temperatures compared to those with non-malarial fever and controls.

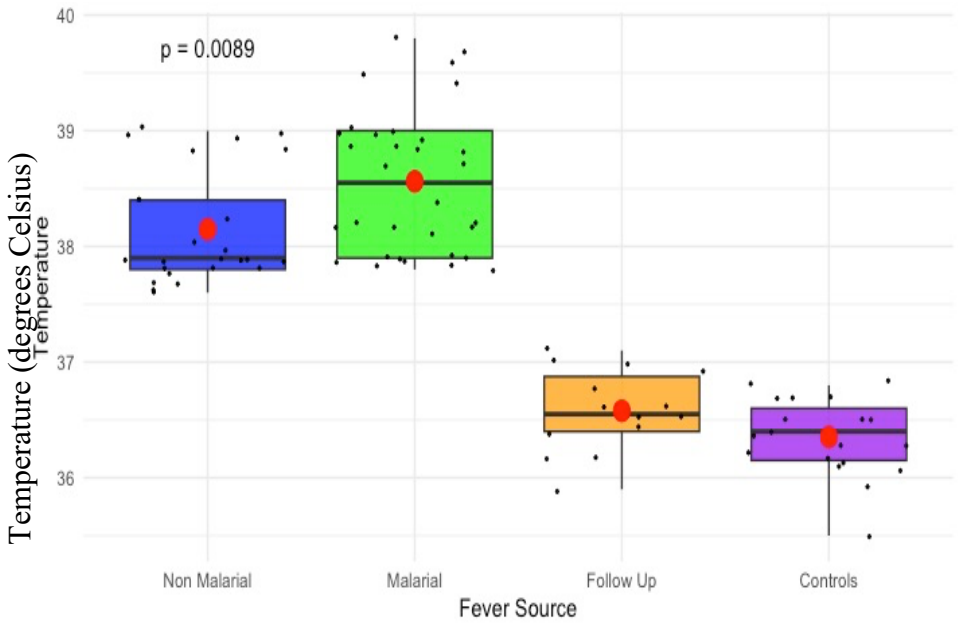

Figure 2: A Box plot of temperature distribution, averages, median and percentiles in malaria fever, non-malarial fever, follow-up and controls in paediatrics presenting with fever and those in without in Blantyre, Malawi

Laboratory results and demographics

Children with malarial fever exhibited distinct clinical profiles compared to those with non-malarial fever. *Table 1* shows demographics, baseline laboratory results and previous medical history for the malarial fever, controls and non-malarial fever. The test of statistical significance was Kruskal Wallis with *df=3*, based on Shapiro result which demonstrated non-normally distributed data. Malarial fever was associated with lower average platelet counts and haemoglobin levels. Mean glucose was higher in malarial fever although the differences in glucose levels were not statistically significant. Laboratory results indicated a significant variation in platelet counts (p=0.02) between the groups, with the control group showing the highest counts. While differences were also noted in WBCs, glucose, and RBCs across the groups, these variations did not achieve statistical significance. WBCs and RBCs count were elevated in non-malarial fever. There were significant differences in

haemoglobin (p-value=0.01) across groups with malarial fever having the lowest value. There was a significant difference between the number of participants that received antimalarials in the past 3 months (p=0.02). The malarial fever group had the highest number of participants who received antimalarials in the past 3 months.

Table 1: Demographics and baseline laboratory results of paediatric patients with or without fever in Blantyre, Malawi

	Non-Malarial Fever(n=25)	**Malaria (n=32)**	**Malaria Follow Up (n=14)**	**Control (n=19)**	**P value across group**
Demographics					
Average age (median (interquartile range))	21 (10-27)	24 (11-29)	19 (9-25)	21(10-27)	0.1
Male gender (n/group total)	9/25	17/32	11/14	7/19	N/A
Nutritional status					
Weight (mean, (SD))	12.08(3.4)	12.07(2.6)	18.45(23.6)	10.62(2.27)	0.1
Height (mean, (SD))	92.72(15.5)	98.84(12.54)	96.0(13.8)	84.68(14.95)	0.2
Laboratory Results					
Haemoglobin g/dL(mean, (SD))	11.2(1.2)	10.48(2.3)	11.01(2.3)	11.41(1.4)	**0.01****
Red blood cells count *10^3 (mean, (SD))	4.49(0.9)	4.25(0.8)	4.28(0.78)	4.8(0.7)	0.3

White blood cells, g/dL(mean, (SD))	9.34(3.6)	9.15(4.4)	9.07(3.7)	8.89(3.4)	0.9
Platelets (mean, (SD))	233.6(123.2)	207.3(114.5)	229.1(65.1)	308.8(112)	**0.02****
Glucose mg/dL (mean, (SD))	104.5(20)	104.6(18.9)	115.9(11.7)	102.2(15.8)	0.1
3 months medical report					
Antibiotics [n/N(%)]	[17/25(68)]	[14/32(44)]	[3/14(21)]	[0/19(0)]	0.2
Antimalarials[n/N(%)]	[1/25(44)]	[7/32(22)]	[6/14(42)]	[0/19(0)]	**0.02****
Hospital admission[n/N(%)]	[1/25(21)]	[1/32(0)]	[0/14(0)]	[0/19(0)]	0.4
Immunisation history					
Rotavirus[n/N(%)]	[23/25(92)]	[32/32(100)]	[14/14(100)]	[17/19(86)]	0.1
Pneumococcal [n/N(%)]	[25/25(100)]	[32/32(100)]	[14/14(100)]	[17/19(86)]	0.2

** indicates a significant difference, Test of significance: Kruskal Wallis, df=3

Clinical manifestations in malaria, non-malarial fever and controls

Further to laboratory results, a comparison of clinical features was carried out to further distinguish malarial fever from non-malarial fever. In particular, spleen palpability, vomiting, diarrhoea, shortness of breath (SOB) and cough were recorded in all groups. *Table 2* shows the frequencies of the clinical symptoms. The most prevalent clinical presentation of both malaria fever and non-malarial fever were cough, vomiting and diarrhoea. Malarial fever was associated with higher spleen palpability than non-malarial fever and controls.

Table 2: Frequency of clinical symptoms in malaria fever, non-malarial fever, controls and follow-up

Variable	Malarial fever (n=32)	Non-malarial fever (n=25)	Controls (n=19)	Malarial follow up (n=14)
Cough	18	16	0	1
Diarrhea	7	8	0	1
SOB	1	0	0	0
Spleen grade	16	2	0	1
Vomiting	15	13	0	1

Correlation of malaria parasite count with haematology results, Temperature, glucose and pulse rate

Figure 3, 4, 5 and *6* shows how malaria parasite counts correlated with different clinical measured parameters. The correlation of each variable and the number of parasites was determined using the Pearson correlation coefficient

(r). The coefficient was calculated together with p values using either Kruskal Wallis or two-way ANOVA based on data normality. The study used Graph Pad prism (version 10) to calculate linear regression and Pearson's correlation coefficient. Even though there was a positive correlation between different haematological and clinical assessments, none of these showed a significant correlation.

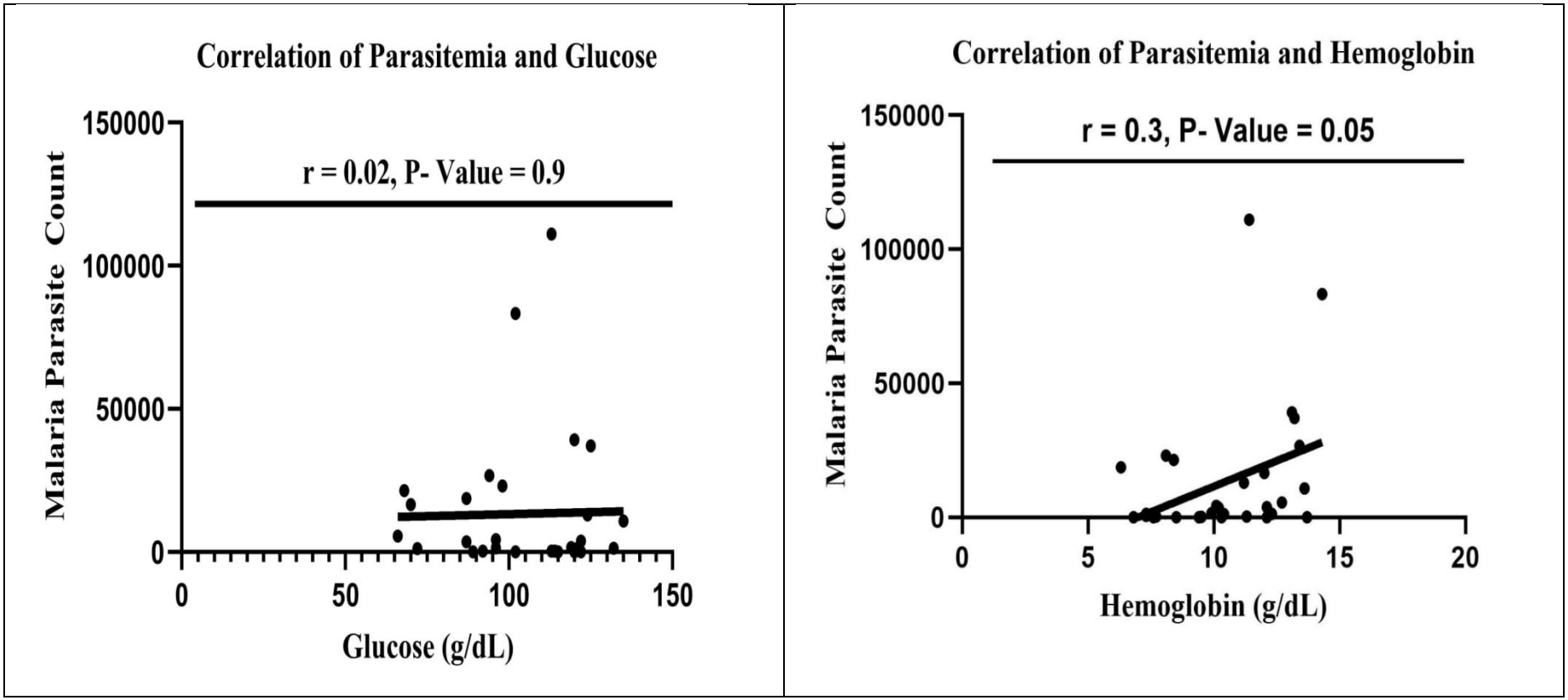

Figure 3: Correlation of parasitaemia to Glucose and Haemoglobin in paediatrics with malarial fever in Blantyre, Malawi

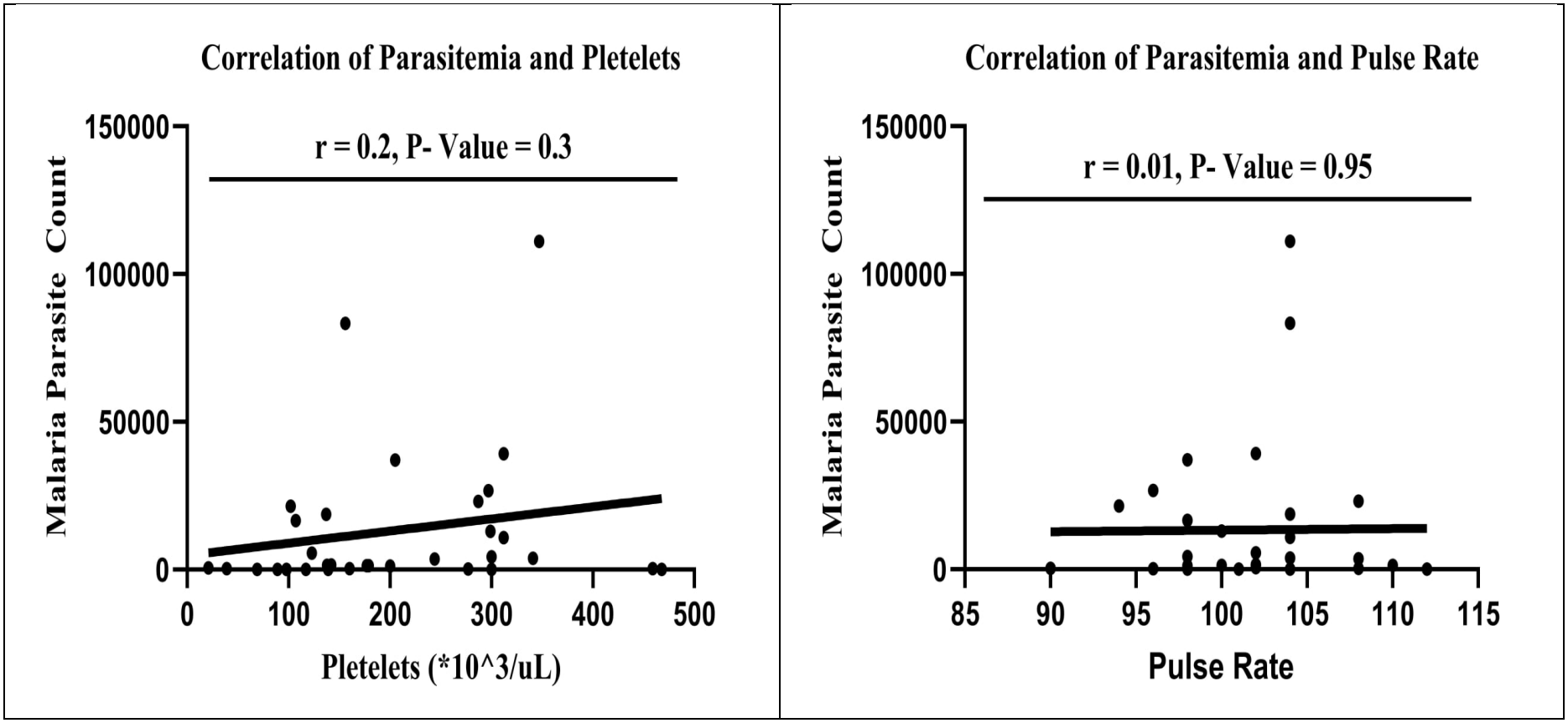

Figure 4: Correlation of parasitaemia to Pulse rate and Platelets in paediatrics with malarial fever in Blantyre, Malawi

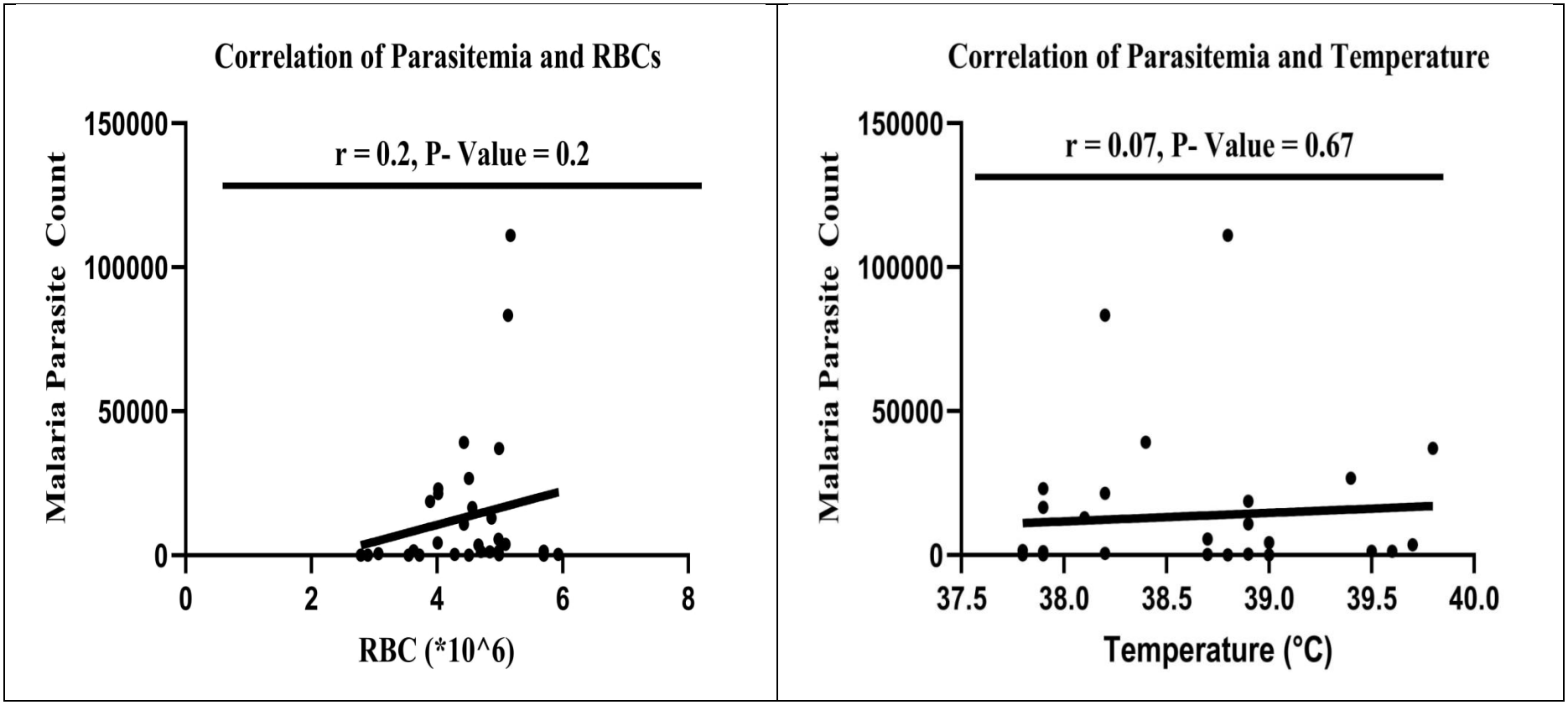

Figure 5: Correlation of malaria parasitaemia and Temperature and RBCs in paediatrics with malarial fever in Blantyre, Malawi

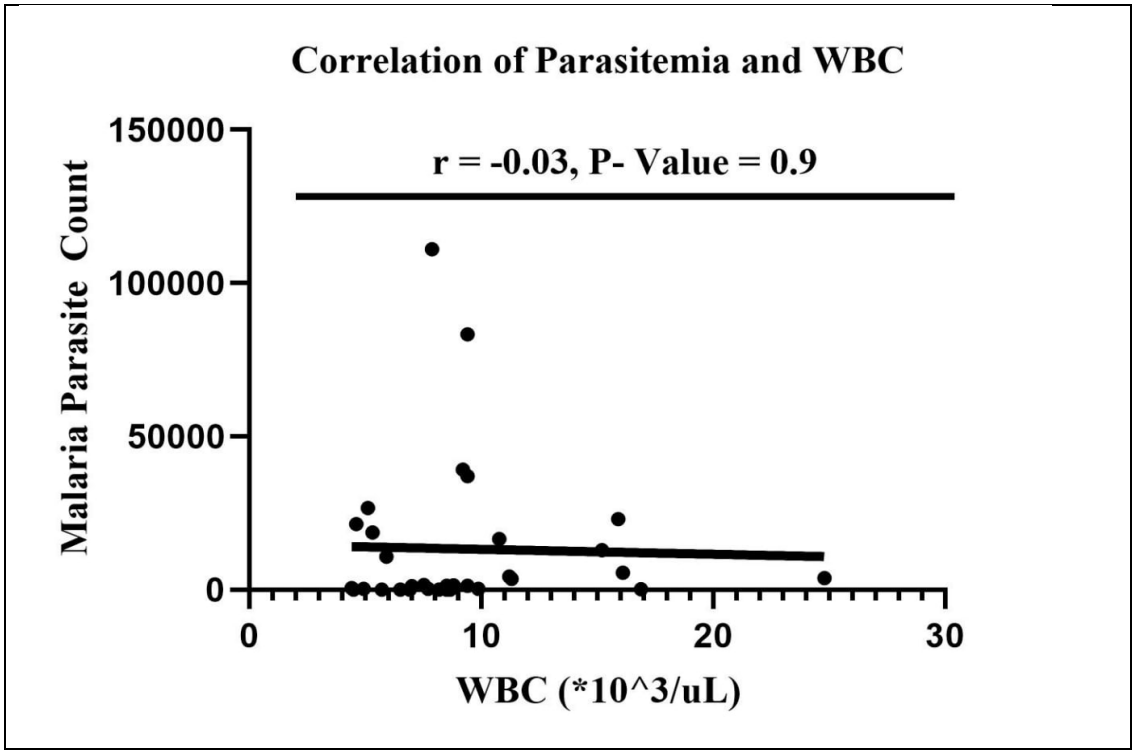

Figure 6: Correlation of parasitaemia and WBC in paediatrics with malarial fever in Blantyre, Malawi

Biomarker profiles

When it comes to the diagnosis and distinction of feverish conditions in paediatric patients, biomarkers can be quite important. This study aimed to profile and compare biomarker levels in children presenting with malarial fever, non-malarial fever and control groups. This was done to identify potential markers that could aid in distinguishing between malarial and non-malaria fever. The biomarker profiles included in this study are C-reactive protein, ICAM-1, Interleukin-6, Interleukin -10, IL-1β, vWF-1 and Ang-2. All the biomarkers were tested using the Luminex multiplex bead assay.

All biomarker results were tested for normality using Shapiro Wilk test. Kruskal Wallis was used for this analysis since data was not normally distributed (Degrees of freedom, *df* =2). *Figure 7* shows Boxplots for (A) CRP: malaria vs. non-malarial fever ($p = 0.09$), (B) IL-10: malarial vs. non-malarial fever ($p \leq 0.0001$), *Figure 8*: (C) vWF-1: malaria vs. non-malaria ($p \leq 0.001$), (D) IL-6: malaria vs. non-malarial fever ($p = 0.04$), *Figure 9*: (E) IL-1 beta: malaria vs. non-malarial fever ($p\leq = 0.01$), (F) ICAM-1: malaria vs. non-malarial fever ($p = <0.005$), and *Figure 10*: (G) ANG-2: malaria vs. non-malarial fever ($p = 0.005$). There were significant differences for IL-10, IL-6, IL-1β, Ang-2 and vWF-1 between the malarial and non-malaria fever groups. Fever from malaria was marked by higher levels of IL-10, IL-6 and increased CRP levels. Non-malarial fever had a remarkable increase of IL-1β, ICAM-1, Ang -2 and vWF-1. None of these markers were increased in controls. The concentrations were converted to log 10 to carter for some concentrations that were too small.

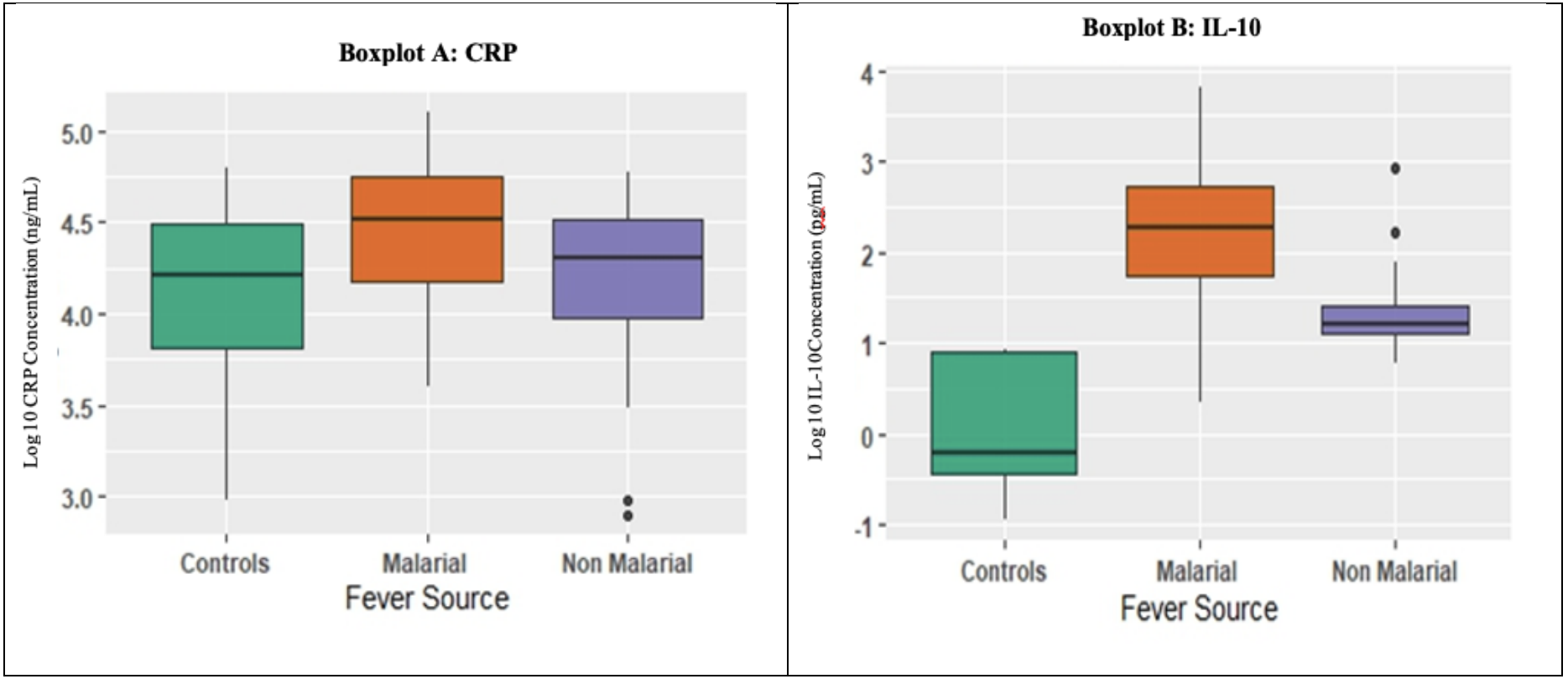

Figure 7: Boxplots of concentration of (A) C-reactive protein and (B) IL-10 across paediatrics with malarial fever, non-malaria fever and controls

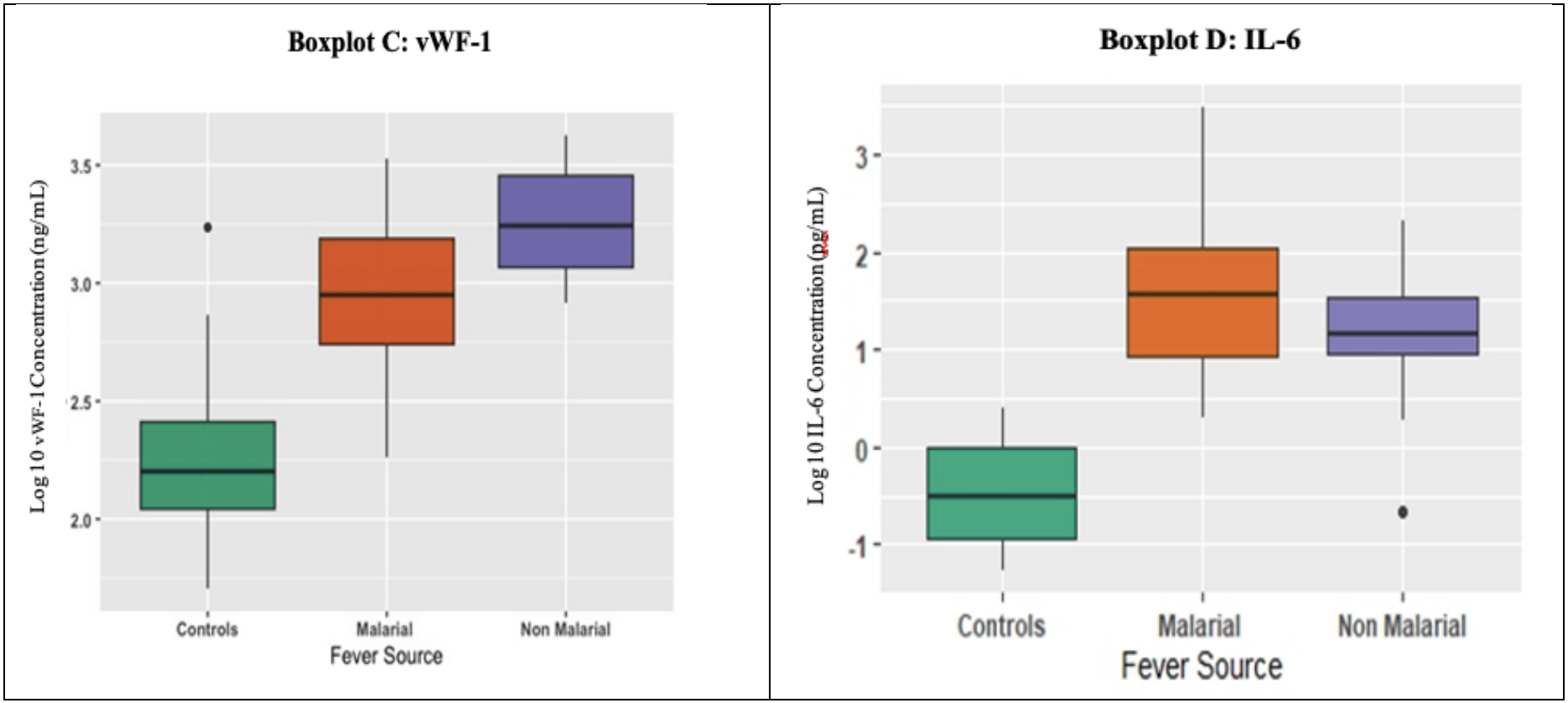

Figure 8: Boxplots of concentration of (C) vWF-1 and (D) IL-6 across paediatrics with malarial fever, non-malaria fever and controls

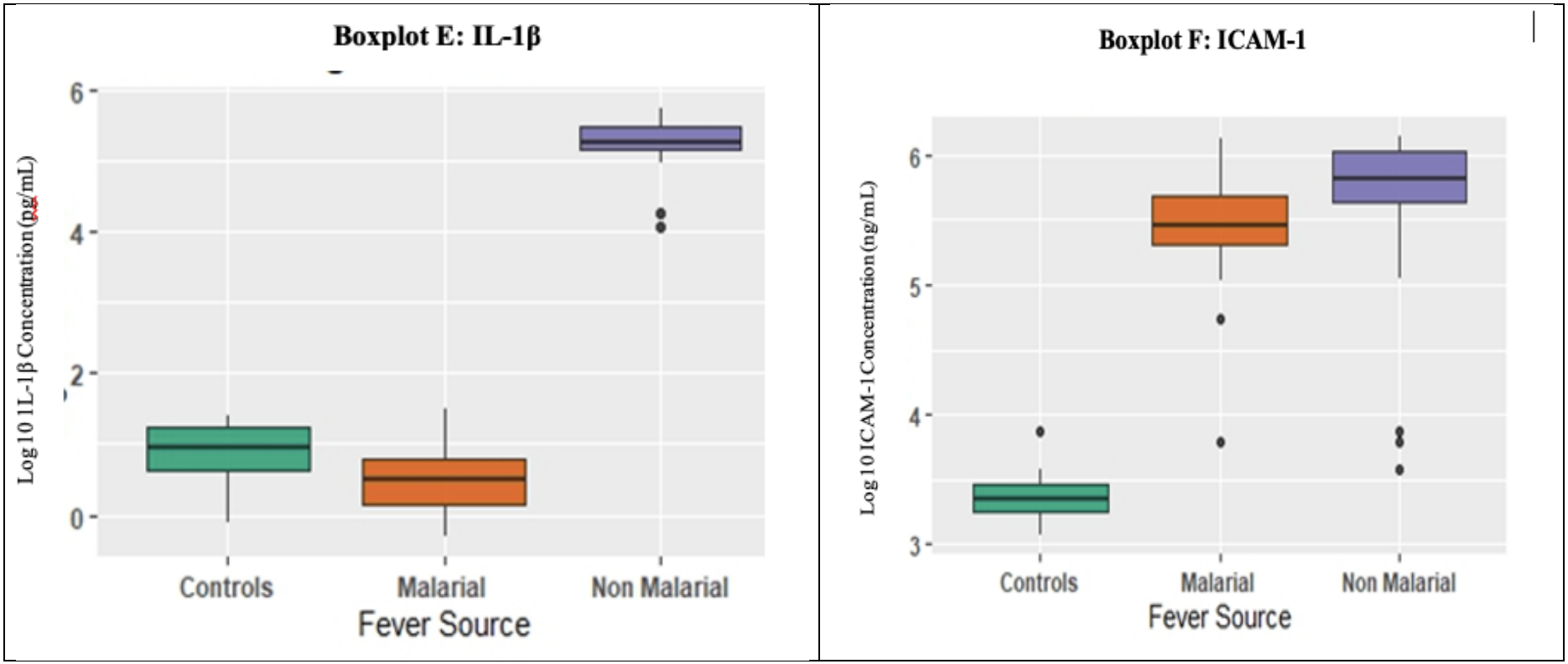

Figure 9: Boxplots of concentration of (E) IL-1β and (B) ICAM-1 across paediatrics with malarial fever, non-malaria fever and controls

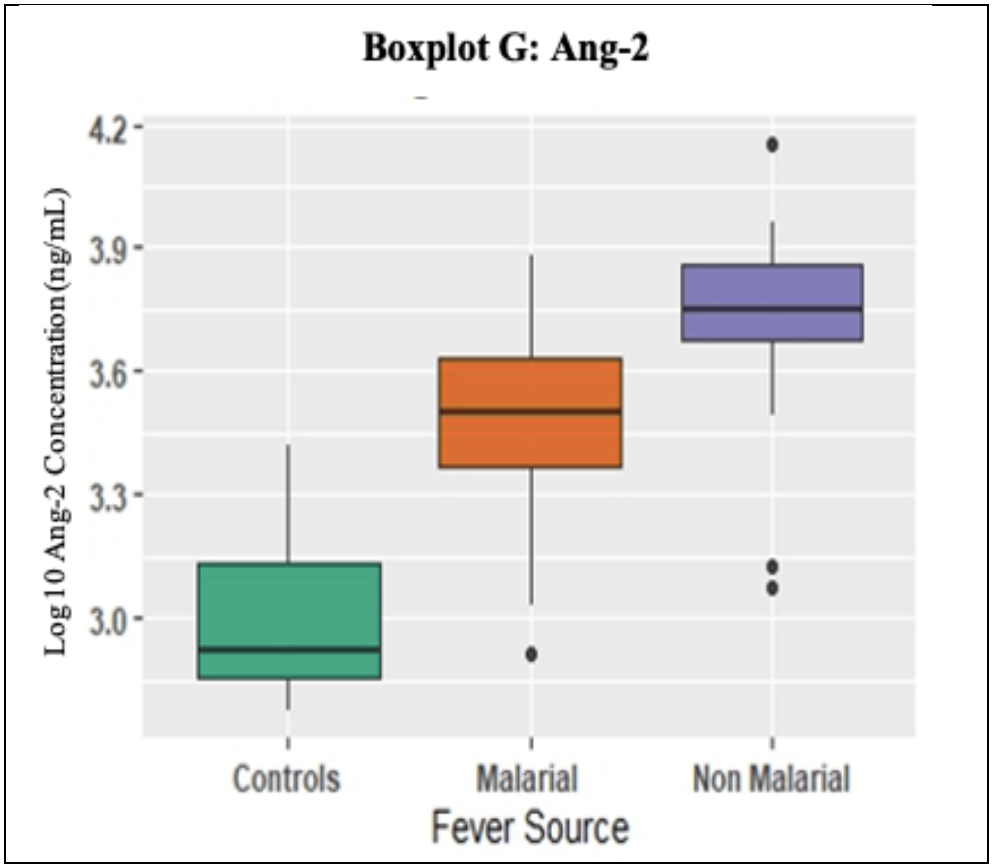

Figure 10: Boxplots of concentration of (G) Ang-2 across paediatrics with malarial fever, non-malaria fever and controls

To find a specific biomarker profile that correctly predicts the fever source, Area under the Receiver Operating Characteristic Curve (AUROC) test was done. This test tells the probability of a marker to correctly identify either malaria fever or non-malarial fever. AUROC are presented with Confidence intervals (CIs) which provide the uncertainty around a sample statistic, such as a mean or proportion. A CI provides a range of values within which we can be confident that the true population parameter lies, typically at a specified confidence level (commonly 95%). A narrow confidence interval indicates a high level of precision and reliability in the estimate, suggesting that repeated sampling would yield similar results. This precision enhances the biomarker's reliability as a diagnostic tool, as it implies consistent performance across different populations or settings. On the other hand, a wide confidence interval reflects greater variability and uncertainty in the estimate, which can diminish the biomarker's reliability. A wide interval suggests that the true effect could vary significantly, making it harder to trust the biomarker's diagnostic capabilities. Therefore, narrower confidence intervals generally enhance the credibility of a biomarker, while wider intervals may raise concerns about its consistency and applicability in clinical practice.

Table 3 summarizes the AUROC (Area Under the Receiver Operating Characteristic Curve) values for each biomarker, along with their corresponding confidence intervals (CI Lower and CI Upper). AUROC values indicate the discriminatory ability of each biomarker in distinguishing between individuals with malarial fever and non-malarial fever, with higher values indicating better discriminatory performance. Confidence intervals offer a range of reasonable

values for the actual AUROC, taking into consideration estimation uncertainty resulting from sampling variability. The comparison of biomarker responses between malaria fever and non-malarial fevers revealed specific patterns indicative of the underlying pathophysiology of each condition. AUROC values are from 0.5 (indicates no discriminative ability) to 1.0 (perfect discrimination), with higher values indicating better performance.

Specifically, as shown in *Figures 8* and *Figure 9*, markers such as IL-6 (AUROC= 0.66; CI=95%), IL-10 (AUROC= 0.81; CI=95%) and CRP (AUROC=0.76; CI=95%) were elevated in individuals with malaria compared to those with non-malarial fevers. Interleukin-6 and Interleukin-10 showed higher levels in malaria-infected individuals and the difference with non-malarial fever was statistically significant. CRP, despite being increased in malarial fever, it had a non-significant difference across the groups and a moderate discriminatory ability. IL-10 had a better discriminatory ability in differentiating malarial fever from non-malarial fever.

All markers of endothelial activation assessed in this investigation demonstrated higher levels in individuals with non-malarial fevers compared to those with malaria as shown in *Table 3*. ICAM-1 (AUROC=0.76; CI=95%), vWF-1 (AUROC=0.71; CI=95%) and ANG-2 (AUROC= 0.79; CI=95%) were elevated in non-malarial fevers, suggesting a distinct mechanism involving endothelial dysfunction in response to bacterial or other non-malarial inflammatory stimuli. IL-1β, an inflammatory cytokine involved in the host response to infections and inflammatory processes, was also elevated in non-malarial fevers than in malarial fever and controls. IL-1β (AUROC=0.9;

CI=95%) showed a better predictability of non-malarial fever than malarial fever.

Table 3: Area Under the Receiver Operating Characteristic Curve (AUROC) with 95% confidence intervals (CI) for various biomarkers in differentiating malarial fever and non-malarial fever

Biomarker	AUROC	CI Lower level	CI Upper level
CRP	0.6	0.5	0.79
IL-1β	0.9	0.6	0.9
ICAM-1	0.76	0.6	0.9
vWF-1	0.71	0.62	0.87
IL-10	0.81	0.7	0.95
Ang-2	0.79	0.67	0.92
IL-6	0.66	0.52	0.8

Support vector machine (SVM) learning for differentiating malarial fever and non-malarial fever

Table 4 presents Support Vector Machine (SVM) Learning for differentiating malarial from non-malarial fever. *Table 4* summarizes how different biomarkers identify a condition (malarial fever or non-malarial fever). Caret package in R Studio was used to calculate Precision, Recall, Specificity and F1 Score for each biomarker. Support vector machine (SVM) learning uses key metrics for evaluating performance including Precision, Recall, Specificity and F1 Score. Precision measures the proportion of true positive predictions among all positive predictions, indicating the model's accuracy when predicting positive cases; high precision is crucial in scenarios where false positives can have serious consequences. Recall, or sensitivity, assesses how well the model

identifies actual positive cases, which is vital in applications like disease detection. Specificity measures the proportion of actual negative cases correctly identified, highlighting the model's ability to minimize false positives. Finally, the F1 Score combines precision and recall into a single metric, providing a balanced evaluation, especially in cases of imbalanced class distributions. Together, these metrics offer a comprehensive view of the SVM model's performance, highlighting its strengths and weaknesses in classifying instances. The results in Table 4 indicate that IL-10 is the most effective biomarker among those tested, with a Precision of 0.86, Recall of 0.75, Specificity of 0.84, and an F1 Score of 0.80. This suggests that IL-10 is both accurate and reliable in identifying malarial cases, which can make it a valuable tool in clinical diagnostics. IL-1β showed a good discriminatory effect and had a higher score for correctly identifying non-malarial fever. In this case, a finding of low IL-1β and high IL-10 in paediatric fever may suggest malaria. All the other markers had lower F1 values and hence had poor diagnostic accuracy.

Table 4: Support Vector Machine (SVM) Learning for differentiating malarial fever and non-malarial fever

Biomarker	Precision	Recall	Specificity	F1 Score
CRP	0.64	0.56	0.60	0.60
IL-1β	0.7	0.64	0.80	0.76
ICAM-1	0.36	0.31	0.28	0.33
vWF-1	0.43	0.38	0.36	0.40
IL-10	0.86	0.75	0.84	0.80
Ang-2	0.29	0.25	0.20	0.27
IL-6	0.64	0.56	0.60	0.60

Discussion

Fever is a naturally occurring adaptive mechanism and forms part of the immune system's inflammatory response. It is characterised by an unnatural rise in body temperature that takes place in response to certain physiological alterations regulated and mediated by the Central Nervous System (CNS) (Bakalli et al., 2022; Madut et al., 2021). Herlihy et al. (2016) indicated that fever is interpreted as a temperature of 38 degrees Celsius (rectal or tympanic), 37.5 degrees Celsius (oral) or 37.5 degrees Celsius (axillary). This fever response aims to provide an unfavourable environment for the infecting pathogen and hence aid in its clearance.

For this study, paediatrics aged between 6 and 60 months were classified as having malarial fever or not based on the Rapid Malaria test and Microscopy. Luminex was used to identify profiles for the biomarkers in malarial and non-malarial fevers. Haematological results and demographics were collected from the study enrolment forms.

Haematological and clinical features associated with malarial fever and non-malarial fever

Temperature

A fever is defined as a rise in body temperature that is more than the typical daily range (Herlihy et al., 2016). For this study, an axillary temperature of ≥37.5 was used to define fever. This is in agreement with (Plucinski et al., 2020) ,who suggested that the lower limit to define fever is 37.5 degrees Celsius based on Mozambican febrile patients. Numerous other studies have used a temperature of ≥37.5 to define fever (Baltzell et al., 2019). Identification of

fever by using temperature is the first clinical feature used to suggest a diagnosis.

In *Figure 2*, the findings of this study showed that there was a significant difference (p=0.0089) in temperature across malarial fever, non-malarial fever and controls. Malarial fever patients presented with the highest mean temperature. Similarly, studies have shown that temperature is much higher in malaria fever than in non-malarial fevers (Baltzell et al., 2019; Plucinski et al., 2020). The temperature differences indicate a different underlying pathophysiological activity in malarial and non-malarial fever with malaria causing a release of more pro-inflammatory cytokines. These findings are crucial for clinical practice as they highlight the importance of temperature measurement in the initial differential diagnosis of febrile illnesses. Higher temperatures in children living in endemic regions can be used to suggest malarial fever. A study by *(Okoro et al., 2020)* in Nigerian children with fever suggested that temperature at admission can be used as a feature to identify malarial fever from non-malarial fever with higher temperature indicating malaria. This implies that higher temperatures during a hospital visit are indicative of malarial fever and can provide guidance for the treatment of pediatrics patients.

Hematological features associated with malaria and non-malarial fever

Paediatric patients with malarial fever demonstrated low mean platelet and mean haemoglobin counts with significance levels at p=0.02 and p=0.01 respectively as shown in *Table 1*. These findings agree with previous findings that platelet (McMorran et al., 2009; Morrell, 2014) and haemoglobin (Antwi-(Antwi-Baffour et al., 2023) were significantly lower in malarial fever. This

occurrence was also noted in Ethiopian children attending Sibu Sire Health Centre(Bayisa & Dufera, 2022) . The decrease in platelets is due to peripheral destruction of platelets or the release of unmatured platelets (Adly et al., 2015; Golwala et al., 2016; Leal-Santos et al., 2013). In a related study, platelets were found elevated in bacterial and viral infections than in malarial fever in Sudan children (Bilal et al., 2016). Platelets have an important role in pathogen binding and subsequent reduced levels can mark malarial fever in paediatrics presenting with fever. Haemoglobin is reduced in malaria fever because of the anaemia resulting from the sequestration of infected red blood cells for destruction in the spleen. This also leads to an inflamed spleen which was common in malarial fever in this study. In agreement with this, studies done in Ghana and Sri Lanka respectively found that a palpable spleen grade was one of the clinical factors associated with malaria in paediatrics and adults (Kularatne et al., 2023; Osei-Kwakye et al., 2013). There was a significant difference in the number of paediatrics that received antimalarials ($p=0.02$) in the past three months. Paediatrics who were on malarial treatment in the past three months had presented more with malaria and this can be evidence of re-infection. This could be due to several factors, including incomplete treatment, drug resistance, or high exposure to malaria vectors in endemic areas, leading to recurrent infections despite previous treatment. The haematological and clinical features give a picture of the fever source with high spleen palpability and low platelet and haemoglobin suggesting malarial fever.

Correlation of malaria parasite count to different clinical results

In terms of the correlation of clinical tests to malaria parasite count in *Figure 3* and *Figure 4*, Haemoglobin ($r=0.3$), RBC ($r = 0.2$) and platelets ($r =$

0.2) demonstrated a moderate correlation with parasite density. This suggests that as the parasite count increases, there is a tendency for haemoglobin, platelets and RBC levels to decrease, albeit not significantly. The decline in RBC and haemoglobin could be attributed to the haemolysis of infected red blood cells (Kularatne et al., 2023; Osei-Kwakye et al., 2013), a common occurrence in malaria-infected individuals and it positively correlates with the parasite count. In *Figure 6*, White blood cell count against malaria parasite count have a negative correlation, which suggests that WBCs are not the main players in malaria clearance and cannot be used to determine parasitaemia.

Biomarkers in malarial fever and non-malarial fever

It is known that cytokine dysregulation plays a role in the pathological processes of infectious diseases like malaria (Davenport et al., 2016). Malarial fever and non-malarial fever present distinct clinical and immunological profiles, which were reflected in the biomarker responses observed in this study. Malaria typically manifests with cyclical febrile episodes coinciding with the parasite's life cycle. These febrile episodes are usually followed by a range of systemic symptoms, including chills, sweating, headache, and myalgia, which are linked to the production of cytokines and other inflammatory mediators in response to parasite invasion and replication within red blood cells (Ong'echa et al., 2011; Zdrodowska et al., 2006).

Biomarkers predicting malarial fever

In malarial fever, the following biomarkers IL-6 ($p=0.04$, AUROC= 0.66, CI=95%), IL-10 ($p=\leq 0.0001$, AUROC= 0.81, CI=95%) and CRP ($p=0.09$, AUROC=0.76, CI=95%) demonstrated a better discriminatory ability of

malarial fever than the markers of endothelial activation as shown in *Table 3.* In terms of concentration, *Table 3*, *Figure 7* and *Figure 8*, showed that IL-6 and IL-10 demonstrated higher levels in malaria-infected individuals and the difference with non-malarial fever was statistically significant. CRP had a non-significant difference across the groups. IL-10 had a better discriminatory ability to differentiate malarial fever from non-malarial fever.

To differentiate bacterial and non-bacterial infections in individuals with fever, C reactive protein (CRP), a measure of inflammation, has been the subject of substantial research (N. Y. Ngwengi et al., 2020). In this study, CRP concentration difference between malaria and non-malarial fever was p= 0.09, this indicates a non-significant difference. This finding agrees with (Bertoli et al., 2021) where CRP levels were higher in malarial fever but it was not able to distinguish malarial fever from bacterial fever and the difference was insignificant. CRP has been widely utilised to distinguish between viral and bacterial fever (Lubell et al., 2015), where it correctly identifies bacterial infections (Nijman et al., 2014) from viral infections. However, studies in Ghana found that the role of CRP in excluding malarial fever from other sources of fever is not significant claimed that the mean CRP levels of bacterial and malaria infections do not differ significantly (Y. Ngwengi et al., 2023). It was also established that compared to a viral illness, mean CRP were substantially higher in cases of malaria or bacterial infection, thus this suggests that CRP can not be used as a reliable biomarker in distinguishing malarial and bacterial infections. These results concur with this current study's findings, in which CRP levels were higher in malarial fever than in non-malarial fever but the differences were not significant. The non-significant differences in CRP can be

due to the overlap of immune responses between malarial fever and non-malarial fever. This suggests that CRP elevation is not specific to either malarial or non-malarial fever and therefore cannot be utilised as a biomarker to differentiate malarial fever from non-malarial fever.

For this study, IL-10 ($p=\leq 0.0001$, AUROC= 0.81, CI=95%) and IL-6 ($p=0.04$, AUROC= 0.66, CI=95%) were higher in malarial fever than in controls and non-malarial fever and the differences were of statistical significance (*Figure 7, Figure 8* and *Table 3*). The equilibrium between the pro- and anti-inflammatory responses is preserved by IL-10 (Popa & Popa, 2021). Studies in malaria fever have demonstrated that IL-10 increases in relation to malaria severity (Sornsenee et al., 2023). IL-10 has also been shown to influence the growth of malaria parasites in murine malaria (Freitas do Rosário et al., 2012). Research combining viral, bacterial and malarial infections showed that IL-10, when applied in a classification tree signature, can reliably classify patients into viral, bacterial and malarial aetiologies, with an overall 96 and 86% specificity and sensitivity, respectively (Valim et al., 2016). This agrees with the finding of this study where IL-10 had AUROC of 0.81, demonstrating a higher discriminatory ability of malarial fever from non-malarial fever. This demonstrates that increased IL-10 measured in fever patients predicts malaria infection.

Inflammatory cytokines, such as Interleukin-6, are released by the immune cells during blood-stage malaria infection. To stop the parasite from growing and to eradicate it, IL-6 is essential. Many studies have studied IL-6 in bacterial infections (Chiaretti et al., 2013; Tanaka & Kishimoto, 2012) and viral fever and Covid-19 (Kang et al., 2015). Studies in non-malarial fever have

demonstrated that IL-6 performs better in differentiating viral and bacterial infections with an AUROC of 0.93 (Valim et al., 2016). Scarce data is available on the comparison of IL-6 on differentiating the cause of fever. With a significant difference between malarial and non-malarial fever and AUROC= 0.66, this study suggests that IL-6 has a moderate influence in differentiating the source of fever.

Biomarkers predicting non-malarial fever

Markers of endothelial activation demonstrated higher levels in individuals with non-malarial fevers compared to those with malaria fever. In *Figure 8, Figure 9*, *Figure 10* and *Table 3*; ICAM-1 (p=≤0.005, AUROC=0.76, CI=95%), vWF-1 (p =≤0.001, AUROC=0.71, CI=95%) and Ang-2 (p=0.005, AUROC= 0.79, CI=95%) were elevated in non-malarial fevers, suggesting a distinct mechanism involving endothelial dysfunction in response to bacterial or other non-malarial inflammatory stimuli.

Data are scarce on the usage of levels ICAM-1, vWF-1, and Ang-2 in the differential diagnosis of malarial and non-malarial fever. However, higher levels of ICAM-1, vWF-1, and Ang-2 have been observed in association with increased severity of infectious diseases (Laurent et al., 2022) such as in viral infections such as COVID-19 patients (Alay & Laloglu, 2021), and *Mycobacterium avium* infection (Ma et al., 2020). A study by Valim *et al*., (2016) found that vWF-1 works better in differentiating bacterial and viral infections, with higher levels indicating viral infections. However, other studies have demonstrated that vWF-1 increased in bacterial infections and also aids in bacterial pathogenesis (Bui et al., 2020; Steinert et al., 2020; Suurbaar et al., 2022). In this study, ICAM-1 (p=≤0.005, AUROC=0.76, CI=95%) and Ang-2*;*

p=0.005, AUROC= 0.79, CI=95%) have been shown to have a better discriminatory effect on malarial fever and non-malarial fever with higher levels suggesting non-malarial fever. This indicates that there is more endothelial activation in paediatrics with non-malarial fever than in those with malarial fever.

IL-1β, a cytokine that promotes inflammation and aids in the host's reaction to infections and inflammatory processes, was also elevated in non-malarial fevers, indicative of a robust inflammatory milieu characteristic of bacterial or other non-malarial infectious agents. In a study of malaria-infected children in Kenya, children with bacterial infection had higher IL-1β (Davenport et al., 2016) than those with malaria. In infectious diseases, sepsis can lead to fever; a study of Malawian children found that IL-1β was the best biomarker to predict non-malarial sepsis, improving sepsis diagnosis in malaria-endemic areas (Baltzell et al., 2019). This relates to the current findings where IL-1β (p=≤0.01, AUROC=0.9, CI=95%) showed higher levels in non-malarial fever than in malarial fever and had a better discriminatory value.

Utility of machine learning in differentiating malarial and non-malarial fever

This study used Support Vector Machines (SVMs) to further distinguish malaria from non-malarial fevers using a range of biomarkers. In *Table 4*, Interleukin-10 demonstrated an F1 score of 0.80, indicating a balanced strength in both precision and recall and correctly identifying malarial cases. Interleukin-1β achieved an F1 score of 0.76 and demonstrated a good discriminatory ability of non-malarial fever from malarial fever. Conversely,

CRP and Interleukin-6 exhibited a more moderate performance, reflected in their F1 scores of 0.60 each. These results show the necessity of proper biomarker selection in SVM-based diagnostic models. Biomarkers with higher F1 scores, like IL-10 and IL-1β have the potential to significantly improve the accuracy of clinical decision-making when differentiating malaria. These results of SVM agree with Baltzell et al. (2019), where IL-1β correctly identified non-malarial sepsis. This cross-study consistency emphasises how strong and accurate IL-1β can be a biomarker for differentiating between febrile diseases. Furthermore, the modest performance of IL-6 and C-reactive protein indicates that although they might be useful in this particular diagnostic context but not as effective as IL-10 and IL-1β.

Mechanisms behind variability of biomarkers in malarial fever and non-malarial fever

The variability in biomarker performance can largely be attributed to the differences in immune responses to malaria compared with other types of fever causing infections. Malaria, caused by the Plasmodium parasite, primarily induces a Th1/Th2 mixed immune response with significant involvement of regulatory cytokines like IL-10, which modulate inflammation and parasite growth (Edwards et al., 2023). In contrast, bacterial and viral infections often trigger a more intense inflammatory response involving pro-inflammatory cytokines like IL-6 and IL-1β, as well as endothelial markers like ICAM-1 and vWF-1 (Ma et al., 2020). In bacterial and viral infections, endothelial activation and dysfunction are more pronounced due to the direct effects of toxins or pathogens on the vascular system, leading to the elevated levels of ICAM-1,

vWF-1, and Ang-2 seen in non-malarial fevers (Tuyen et al., 2020). These markers are not typically as elevated in malaria, where the immune response is more focused on parasite clearance rather than endothelial injury.

Chapter summary

In summary, the study investigated whether different biomarkers could help distinguish between malaria fever and non-malarial fever in paediatric patients. Temperature was higher in malarial fever than in non-malarial fever. Haematological results demonstrated reduced haemoglobin and platelet count in malarial fever than in non-malarial fever and controls. The findings showed distinct patterns in biomarker levels between the groups. Children with malaria had elevated levels of IL-6 and IL-10 compared to the other groups. Notably, IL-10 appeared to be the most promising biomarker for differentiating malaria from non-malarial fever. All markers of endothelial activation were increased in non-malarial fever. IL-1β, ICAM-1, Ang-2 and vWF=1 were increased in non-malarial fever and demonstrated a good discriminator ability between malarial and non-malarial fever. Finally, a machine learning technique (Support Vector Machine) was used to analyse the biomarker data. This analysis confirmed that IL-10 and IL-1β are the most effective biomarkers for differentiating malaria fever from non-malarial fever. These findings highlight the potential of using specific biomarker profiles to improve diagnosis of febrile illnesses in children.

CHAPTER FIVE

SUMMARY, CONCLUSIONS AND RECOMMENDATION

Summary

The study aimed to explore the use of serum biomarkers to distinguish malaria from non-malaria fever in paediatric patients in Blantyre, Malawi. This was done by analysing serum profiles of specific biomarkers that are expressed in malarial fever and non-malarial fever. The study aimed to improve diagnostic accuracy and reduce unnecessary antibiotic use, particularly in malaria-endemic regions where fever presents a diagnostic challenge due to overlapping clinical features of different infectious diseases. Temperature was higher in malarial fever than in non-malarial fever. Haematological results demonstrated reduced haemoglobin and platelet count in malarial fever than in non-malarial fever and controls. It was hypothesized that serum biomarkers will be expressed differently between malaria and non-malaria fever. The Luminex bead assay method was utilized to measure the biomarkers in the study. IL-10, IL-1β, ICAM-1, Ang-2, vWF-1, and IL-6 were identified as biomarkers with notable variations in their levels among malarial and non-malarial fever. Among these, IL-10 and IL-1β correctly predict fever sources with IL-10 increased in malarial and IL-1β increased in non-malarial fever. Additionally, all markers of endothelial activation such as ICAM-1, Ang-2 and vWF-1 were elevated in non-malarial fever than in malaria cases and demonstrated moderate discriminatory ability.

Conclusion

In paediatric patients presenting with fever, IL-10 and IL-1β correctly differentiates malarial fever from non-malarial fever with IL-10 levels increased in malarial fever and IL-1β increased in non-malarial fever. The utility of these can aid in the quick diagnosis of aetiology of infections that cause fever. This study on serum biomarkers in Malawian paediatric patients has provided novel insights by identifying specific biomarkers, such as IL-10, IL-1β, ICAM-1, ANG-2, vWF-1, and IL-6, that exhibit significant differences in expression levels across malaria and non-malarial fever. In addition to the biomarkers, malarial fever presents with lower haemoglobin and platelets with increased temperature compared to non-malarial fever. These findings offer promising prospects for enhancing diagnostic precision in paediatric infectious diseases.

Recommendations

In order to confirm the diagnostic effectiveness of IL-10 and IL-1β in differentiating malarial fever from non-malarial fever, further extensive investigations including a wider range of paediatric patients from several sites are required. These investigations would validate the results and demonstrate the dependability of IL-10 and IL-1β biomarkers in various clinical contexts. Additionally, evaluating the cost-effectiveness and clinical implications of integrating IL-10 and IL-1β testing into routine practices is critical, as understanding their practical and economic impacts will guide their adoption. Developing and implementing diagnostic algorithms or decision-support tools that integrate IL-10 and IL-1β with other clinical and laboratory parameters

could significantly enhance diagnostic accuracy and reliability, improving the differentiation of fever types in paediatric patients. Extensive research on the processes behind the distinct regulation of IL-10 and IL-1β in malarial and non-malarial fever may provide a more profound understanding and improve diagnostic methodologies. The wider implementation would be supported by interacting with professional associations to push for these biomarkers' inclusion in clinical guidelines. To ensure that the diagnostic benefits of IL-10 and IL-1β are broadly applicable and useful, it is imperative to assess the transportability of these findings to diverse geographical regions and healthcare settings.

Suggestions for further research

Further research should be done to investigate the longitudinal dynamics of IL-10 and IL-1β in paediatric patients with malaria and co-infections. This longitudinal study could focus on monitoring changes in biomarker levels over time during the course of treatment and recovery.

REFERENCES

Acero, C. T., Ramírez-Montoya, J., Velasco, M. C., Avilés-Vergara, P. A., Ricardo-Caldera, D., Duran-Frigola, M., Quintero, G., Cantero, M. E., Rivera-Correa, J., Rodriguez, A., & Yasnot-Acosta, M. F. (2022). IL-4, IL-10, CCL2 and TGF-β as potential biomarkers for severity in Plasmodium vivax malaria. *PLOS Neglected Tropical Diseases*, *16*(9), e0010798. https://doi.org/10.1371/journal.pntd.0010798

Adedokun, S. T., & Yaya, S. (2020). Childhood morbidity and its determinants: evidence from 31 countries in sub-Saharan Africa. *BMJ Global Health*, *5*(10), e003109. https://doi.org/10.1136/bmjgh-2020-003109

Adly, A. A. M., Ragab, I. A., Ismail, E. A. R., & Farahat, M. M. (2015). Evaluation of the immature platelet fraction in the diagnosis and prognosis of childhood immune thrombocytopenia. *Platelets*, *26*(7), 645–650. https://doi.org/10.3109/09537104.2014.969220

Akwii, R. G., Sajib, M. S., Zahra, F. T., & Mikelis, C. M. (2019). Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology. *Cells*, *8*(5), 471. https://doi.org/10.3390/cells8050471

Alay, H., & Laloglu, E. (2021). The role of angiopoietin-2 and surfactant protein-D levels in SARS-CoV-2-related lung injury: A prospective, observational, cohort study. *Journal of Medical Virology*, *93*(10), 6008–6015. https://doi.org/10.1002/JMV.27184

Alcaide, M. L., Rodriguez, V. J., Brown, M. R., Pallikkuth, S., Arheart, K., Martinez, O., Roach, M., Fichorova, R. N., Jones, D. L., Pahwa, S., &

Fischl, M. A. (2017). High Levels of Inflammatory Cytokines in the Reproductive Tract of Women with BV and Engaging in Intravaginal Douching: A Cross-Sectional Study of Participants in the Women Interagency HIV Study. *AIDS Research and Human Retroviruses*, *33*(4), 309–317. https://doi.org/10.1089/aid.2016.0187

Angchaisuksiri, P. (2014). Coagulopathy in malaria. *Thromb Res*, *133*(1), 5–9. https://doi.org/10.1016/j.thromres.2013.09.030

Ansumana, R., Jacobsen, K. H., Gbakima, A. A., Hodges, M. H., Lamin, J. M., Leski, T. A., Malanoski, A. P., Lin, B., Bockarie, M. J., & Stenger, D. A. (2013). Presumptive self-diagnosis of malaria and other febrile illnesses in Sierra Leone. *The Pan African Medical Journal*, *15*. https://doi.org/10.11604/PAMJ.2013.15.34.2291

Antwi-Baffour, S., Mensah, B. T., Johnson, G., Armah, D. N. O., Ali-Mustapha, S., & Annison, L. (2023). Haematological parameters and their correlation with the degree of malaria parasitaemia among outpatients attending a polyclinic. *Malaria Journal*, *22*(1). https://doi.org/10.1186/S12936-023-04710-3

Azad, M. A., & Patel, R. (2024). Practical Guidance for Clinical Microbiology Laboratories: Microbiologic diagnosis of implant-associated infections. *Clinical Microbiology Reviews*, *37*(2). https://doi.org/10.1128/CMR.00104-23

Babigumira, J. B., Gelband, H., & Garrison, L. P. (2017). Cost-Effectiveness of Strategies for the Diagnosis and Treatment of Febrile Illness in

Children. In K. K. Holmes, S. Bertozzi, B. R. Bloom, & P. Jha (Eds.), *Major Infectious Diseases* (3rd ed.). The International Bank for Reconstruction and Development / The World Bank. http://www.ncbi.nlm.nih.gov/books/NBK525175/

Bakalli, I., Klironomi, D., Kola, E., & Celaj, E. (2022). The management of fever in children. *Minerva Pediatr (Torino)*, *74*(5), 568–578. https://doi.org/10.23736/S2724-5276.22.06680-0

Balerdi-Sarasola, L., Parolo, C., Fleitas, P., Cruz, A., Subirà, C., Rodríguez-Valero, N., Almuedo-Riera, A., Letona, L., Álvarez-Martínez, M. J., Valls, M. E., Vera, I., Mayor, A., Muñoz, J., & Camprubí-Ferrer, D. (2023). Host biomarkers for early identification of severe imported Plasmodium falciparum malaria. *Travel Medicine and Infectious Disease*, *54*. https://doi.org/10.1016/J.TMAID.2023.102608

Baltzell, K., Kortz, T. B., Scarr, E., Blair, A., Mguntha, A., Bandawe, G., Schell, E., & Rankin, S. (2019). "Not all fevers are malaria": a mixed methods study of non-malarial fever management in rural southern Malawi. *Rural Remote Health*, *19*(2), 4818. https://doi.org/10.22605/RRH4818

Bayisa, G., & Dufera, M. (2022). Malaria Infection, Parasitemia, and Hemoglobin Levels in Febrile Patients Attending Sibu Sire Health Facilities, Western Ethiopia. *BioMed Research International*, *2022*. https://doi.org/10.1155/2022/6161410

Bertoli, G., Mazzi, C., Ronzoni, N., Silva, R., Spinicci, M., Pozzi, M., Sponga, P., Aiello, A., Ursini, T., Bartoloni, A., Olliaro, P., Bisoffi, Z., &

Buonfrate, D. (2021). C-Reactive Protein for the Early Assessment of Non-Malarial Febrile Patients: A Retrospective Diagnostic Study. *Diagnostics (Basel, Switzerland)*, *11*(9). https://doi.org/10.3390/DIAGNOSTICS11091728

BHARDWAJ, A. N. J. U., & BARAN, D. A. (2023). Biomarkers in HF: How Does the "Weight" Weigh in? *Journal of Cardiac Failure*, *29*(8), 1132–1134. https://doi.org/10.1016/J.CARDFAIL.2023.06.003

Bhattacharya, S., & Munshi, C. (2023). Biological significance of C-reactive protein, the ancient acute phase functionary. *Frontiers in Immunology*, *14*. https://doi.org/10.3389/fimmu.2023.1238411

Bilal, J. A., Gasim, G. I., Karsani, A. H., Elbashir, L. M., & Adam, I. (2016). Malaria Parasite Density Estimation using Actual and Assumed White Blood Cells Count in Children in Eastern Sudan. *Journal of Tropical Pediatrics*, *62*(2), 171–175. https://doi.org/10.1093/tropej/fmv087

Binnicker, M. J. (2015). Multiplex Molecular Panels for Diagnosis of Gastrointestinal Infection: Performance, Result Interpretation, and Cost-Effectiveness. *Journal of Clinical Microbiology*, *53*(12), 3723–3728. https://doi.org/10.1128/JCM.02103-15

Birhanu, Z., Abebe, L., Sudhakar, M., Dissanayake, G., Yihdego, Y. Y. E., Alemayehu, G., & Yewhalaw, D. (2016). Malaria related perceptions, care seeking after onset of fever and anti-malarial drug use in malaria endemic settings of Southwest Ethiopia. *PLoS ONE*, *11*(8). https://doi.org/10.1371/journal.pone.0160234

Bisoffi, Z., Gobbi, F., Buonfrate, D., & den Ende, J. (2012). Diagnosis of Malaria Infection with or without Disease. *Mediterr J Hematol Infect Dis*, *4*(1), e2012036. https://doi.org/10.4084/MJHID.2012.036

Bodaghi, A., Fattahi, N., & Ramazani, A. (2023). Biomarkers: Promising and valuable tools towards diagnosis, prognosis and treatment of Covid-19 and other diseases. *Heliyon*, *9*(2). https://doi.org/10.1016/J.HELIYON.2023.E13323

Bria, Y. P., Yeh, C. H., & Bedingfield, S. (2021). Significant symptoms and nonsymptom-related factors for malaria diagnosis in endemic regions of Indonesia. *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, *103*, 194–200. https://doi.org/10.1016/J.IJID.2020.11.177

Bui, T. M., Wiesolek, H. L., & Sumagin, R. (2020). ICAM-1: A master regulator of cellular responses in inflammation, injury resolution, and tumorigenesis. *J Leukoc Biol*, *108*(3), 787–799. https://doi.org/10.1002/JLB.2MR0220-549R

Calderaro, A., Piccolo, G., & Chezzi, C. (2024). The Laboratory Diagnosis of Malaria: A Focus on the Diagnostic Assays in Non-Endemic Areas. *Int J Mol Sci*, *25*(2), 695. https://doi.org/10.3390/ijms25020695

Çaldıran, F. Y., Çitli, Ş., Çaçan, E., & Deveci, K. (2021). IL-1β, IL-18 and Caspase-1 Levels in Serum as an Early Marker in Familial Mediterranean Fever Patients with Attack and Attack-free Period. *J Contemp Med*, *11*(4), 494–499. https://doi.org/10.16899/jcm.907284

Califf, R. M. (2018). Biomarker definitions and their applications. *Experimental Biology and Medicine*, *243*(3), 213–221. https://doi.org/10.1177/1535370217750088

Caminade, C., McIntyre, K. M., & Jones, A. E. (2019). Impact of recent and future climate change on vector-borne diseases. *Annals of the New York Academy of Sciences*, *1436*(1), 157–173. https://doi.org/10.1111/NYAS.13950

Cantey, J. B., & Lee, J. H. (2021). Biomarkers for the Diagnosis of Neonatal Sepsis. *Clinics in Perinatology*, *48*(2), 215–227. https://doi.org/10.1016/J.CLP.2021.03.012

Cardona-Arias, J. A., Salas-Zapata, W., & Carmona-Fonseca, J. (2020). Systematic review of qualitative studies about malaria in Colombia. *Heliyon*, *6*(5). https://doi.org/10.1016/J.HELIYON.2020.E03964

Chiappini, E., Principi, N., Longhi, R., Tovo, P.-A., Becherucci, P., Bonsignori, F., Esposito, S., Festini, F., Galli, L., Lucchesi, B., Mugelli, A., de Martino, M., & Writing Committee of the Italian Pediatric Society Panel for the Management of Fever in Children. (2009). Management of fever in children: summary of the Italian Pediatric Society guidelines. *Clin Ther*, *31*(8), 1826–1843. https://doi.org/10.1016/j.clinthera.2009.08.006

Chiaretti, A., Pulitanò, S., Barone, G., Ferrara, P., Romano, V., Capozzi, D., & Riccardi, R. (2013). IL-1 β and IL-6 upregulation in children with H1N1

influenza virus infection. *Mediators Inflamm*, *2013*, 495848. https://doi.org/10.1155/2013/495848

Chilanga, E., Collin-Vézina, D., MacIntosh, H., Mitchell, C., & Cherney, K. (2020). Prevalence and determinants of malaria infection among children of local farmers in Central Malawi. *Malaria Journal*, *19*(1), 308. https://doi.org/10.1186/s12936-020-03382-7

Cohee, L. M., Opondo, C., Clarke, S. E., Halliday, K. E., Cano, J., Shipper, A. G., Barger-Kamate, B., Djimde, A., Diarra, S., Dokras, A., Kamya, M. R., Lutumba, P., Ly, A. B., Nankabirwa, J. I., Njagi, J. K., Maiga, H., Maiteki-Sebuguzi, C., Matangila, J., Okello, G., … Chico, R. M. (2020). Preventive malaria treatment among school-aged children in sub-Saharan Africa: a systematic review and meta-analyses. *The Lancet. Global Health*, *8*(12), e1499–e1511. https://doi.org/10.1016/S2214-109X(20)30325-9

Cohen, J. L., Leslie, H. H., Saran, I., & Fink, G. (2020). Quality of clinical management of children diagnosed with malaria: A cross-sectional assessment in 9 sub-Saharan African countries between 2007-2018. *PLoS Medicine*, *17*(9). https://doi.org/10.1371/journal.pmed.1003254

Connon, R., George, E. C., Olupot-Olupot, P., Kiguli, S., Chagaluka, G., Alaroker, F., Opoka, R. O., Mpoya, A., Walsh, K., Engoru, C., Nteziyaremye, J., Mallewa, M., Kennedy, N., Nakuya, M., Namayanja, C., Nabawanuka, E., Sennyondo, T., Amorut, D., Williams Musika, C., … Tenu, F. (2021). Incidence and predictors of hospital readmission in children presenting with severe anaemia in Uganda and Malawi: a

secondary analysis of TRACT trial data. *BMC Public Health*, *21*(1). https://doi.org/10.1186/S12889-021-11481-6

Conroy, A. L., Phiri, H., Hawkes, M., Glover, S., Mallewa, M., Seydel, K. B., Taylor, T. E., Molyneux, M. E., & Kain, K. C. (2010). Endothelium-based biomarkers are associated with cerebral malaria in Malawian children: a retrospective case-control study. *PloS One*, *5*(12). https://doi.org/10.1371/JOURNAL.PONE.0015291

Danis, M. (2023). [Malaria today]. *Medecine Tropicale et Sante Internationale*, *3*(2). https://doi.org/10.48327/MTSI.V3I2.2023.375

Davenport, G. C., Hittner, J. B., Otieno, V., Karim, Z., Mukundan, H., Fenimore, P. W., Hengartner, N. W., McMahon, B. H., Kempaiah, P., Ong'Echa, J. M., & Perkins, D. J. (2016). Reduced parasite burden in children with falciparum malaria and bacteremia coinfections: Role of mediators of inflammation. *Mediators of Inflammation*, *2016*. https://doi.org/10.1155/2016/4286576

Dayal, R., & Agarwal, D. (2016). Fever in Children and Fever of Unknown Origin. *Indian Journal of Pediatrics*, *83*(1), 38–43. https://doi.org/10.1007/S12098-015-1724-4

de Oliveira, C. M. B., Sakata, R. K., Issy, A. M., Gerola, L. R., & Salomão, R. (2011). Cytokines and pain. *Revista Brasileira de Anestesiologia*, *61*(2), 255–265. https://doi.org/10.1016/S0034-7094(11)70029-0

Diema Konlan, K., Amu, H., Konlan, K. D., & Japiong, M. (2019). Awareness and Malaria Prevention Practices in a Rural Community in the Ho

Municipality, Ghana. *Interdiscip Perspect Infect Dis*, *2019*, 9365823. https://doi.org/10.1155/2019/9365823

Dinarello, C. A. (2011). A clinical perspective of IL-1β as the gatekeeper of inflammation. *European Journal of Immunology*, *41*(5), 1203–1217. https://doi.org/10.1002/eji.201141550

Dorado, J. M. B., Dans, L. F., Tan-Lim, C. S. C., Galingana, C. L. T., Panganiban, J. M. S., Rey, M. P., Sanchez, J. T., Zabala, H. S., Aquino, M. R. N., & Dans, A. L. (2024). Frequency Distribution of Pediatric Primary Care Cases in a Rural Site in The Philippines: A Cross-Sectional Study. *Acta Medica Philippina*, *58*(18), 71–78. https://doi.org/10.47895/amp.vi0.8551

Doumbe-Belisse, P., Kopya, E., Ngadjeu, C. S., Sonhafouo-Chiana, N., Talipouo, A., Djamouko-Djonkam, L., Awono-Ambene, H. P., Wondji, C. S., Njiokou, F., & Antonio-Nkondjio, C. (2021). Urban malaria in sub-Saharan Africa: dynamic of the vectorial system and the entomological inoculation rate. *Malaria Journal*, *20*(1). https://doi.org/10.1186/S12936-021-03891-Z

Edwards, C. L., Ng, S. S., de Labastida Rivera, F., Corvino, D., Engel, J. A., de Oca, M., Bukali, L., Frame, T. C., Bunn, P. T., Chauhan, S. B., Singh, S. S., Wang, Y., Na, J., Amante, F. H., Loughland, J. R., Soon, M. S., Waddell, N., Mukhopadhay, P., Koufariotis, L. T., … Engwerda, C. R. (2023). IL-10-producing Th1 cells possess a distinct molecular signature in malaria. *J Clin Invest*, *133*(1), e153733. https://doi.org/10.1172/JCI153733

Edwards, C. L., Ng, S. S., De Labastida Rivera, F., Corvino, D., Engel, J. A., Montes De Oca, M., Bukali, L., Frame, T. C. M., Bunn, P. T., Chauhan, S. B., Singh, S. S., Wang, Y., Na, J., Amante, F. H., Loughland, J. R., Soon, M. S. F., Waddell, N., Mukhopadhay, P., Koufariotis, L. T., … Engwerda, C. R. (2023). IL-10–producing Th1 cells possess a distinct molecular signature in malaria. *Journal of Clinical Investigation*, *133*(4), e169299. https://doi.org/10.1172/JCI169299

Eggimann, P., Que, Y. A., & Rebeaud, F. (2019). Measurement of pancreatic stone protein in the identification and management of sepsis. *Biomarkers in Medicine*, *13*(2), 135–145. https://doi.org/10.2217/bmm-2018-0194

El-Radhi, A. S. (2019). Fever in Common Infectious Diseases. *Clinical Manual of Fever in Children*, 85–140. https://doi.org/10.1007/978-3-319-92336-9_5

Elven, J., Dahal, P., Ashley, E. A., Thomas, N. V, Shrestha, P., Stepniewska, K., Crump, J. A., Newton, P. N., Bell, D., Reyburn, H., Hopkins, H., & Guérin, P. J. (2020). Non-malarial febrile illness: a systematic review of published aetiological studies and case reports from Africa, 1980–2015. *BMC Medicine*, *18*(1), 279. https://doi.org/10.1186/s12916-020-01744-1

Escadafal, C., Geis, S., Siqueira, A. M., Agnandji, S. T., Shimelis, T., Tadesse, B. T., Massinga Loembé, M., Harris, V., Fernandez-Carballo, B. L., Macé, A., Ongarello, S., Rodriguez, W., & Dittrich, S. (2020). Bacterial versus non-bacterial infections: a methodology to support use-case-

driven product development of diagnostics. *BMJ Glob Health*, *5*(10), e003141. https://doi.org/10.1136/bmjgh-2020-003141

Essack, S. Y., Desta, A. T., Abotsi, R. E., & Agoba, E. E. (2017). Antimicrobial resistance in the WHO African region: Current status and roadmap for action. *Journal of Public Health (United Kingdom)*, *39*(1), 8–13. https://doi.org/10.1093/PUBMED/FDW015

Feleke, S. M., Reichert, E. N., Mohammed, H., Brhane, B. G., Mekete, K., Mamo, H., Petros, B., Solomon, H., Abate, E., Hennelly, C., Denton, M., Keeler, C., Hathaway, N. J., Juliano, J. J., Bailey, J. A., Rogier, E., Cunningham, J., Aydemir, O., & Parr, J. B. (2021). Plasmodium falciparum is evolving to escape malaria rapid diagnostic tests in Ethiopia. *Nature Microbiology*, *6*(10), 1289. https://doi.org/10.1038/s41564-021-00962-4

Fitzgerald, E., Ciccone, E. J., Mvalo, T., Chiume, M., Mgusha, Y., Mkaliainga, T. B., Tilly, A. E., Chen, J., Bell, G., Crouse, H., Robison, J. A., & Eckerle, M. (2024). Comprehensive assessment of pediatric acute and inpatient care at a tertiary referral hospital in Malawi: opportunities for quality improvement. *BMJ Paediatrics Open*, *8*(1). https://doi.org/10.1136/BMJPO-2023-002404

Foko, L. P. K., Narang, G., Tamang, S., Hawadak, J., Jakhan, J., Sharma, A., & Singh, V. (2022). The spectrum of clinical biomarkers in severe malaria and new avenues for exploration. *Virulence*, *13*(1), 634–653. https://doi.org/10.1080/21505594.2022.2056966

Freitas do Rosário, A. P., Lamb, T., Spence, P., Stephens, R., Lang, A., Roers, A., Muller, W., O'Garra, A., & Langhorne, J. (2012). IL-27 promotes IL-10 production by effector Th1 CD4+ T cells: a critical mechanism for protection from severe immunopathology during malaria infection. *Journal of Immunology (Baltimore, Md. : 1950)*, *188*(3), 1178–1190. https://doi.org/10.4049/JIMMUNOL.1102755

Frimpong, A., Amponsah, J., Adjokatseh, A. S., Agyemang, D., Bentum-Ennin, L., Ofori, E. A., Kyei-Baafour, E., Akyea-Mensah, K., Adu, B., Mensah, G. I., Amoah, L. E., & Kusi, K. A. (2020). Asymptomatic Malaria Infection Is Maintained by a Balanced Pro- and Anti-inflammatory Response. *Frontiers in Microbiology*, *11*. https://doi.org/10.3389/FMICB.2020.559255

Frimpong, A., Owusu, E. D. A., Amponsah, J. A., Obeng-Aboagye, E., Puije, W. van der, Frempong, A. F., Kusi, K. A., & Ofori, M. F. (2022). Cytokines as Potential Biomarkers for Differential Diagnosis of Sepsis and Other Non-Septic Disease Conditions. *Frontiers in Cellular and Infection Microbiology*, *12*. https://doi.org/10.3389/fcimb.2022.901433

Garcia, L. S. (2010). Malaria. *Clinics in Laboratory Medicine*, *30*(1), 93–129. https://doi.org/10.1016/J.CLL.2009.10.001

García-Hernández, P., Prieto, B., Martínez-Morillo, E., Rodríguez, V., & Álvarez, F. V. (2016). Interleukin-6 in cerebrospinal fluid as a biomarker of acute meningitis. *Annals of Clinical Biochemistry*, *53*(Pt 1), 155–163. https://doi.org/10.1177/0004563215589381

Gaston, R. T., & Ramroop, S. (2020). Prevalence of and factors associated with malaria in children under five years of age in Malawi, using malaria indicator survey data. *Heliyon*, *6*(5), e03946. https://doi.org/10.1016/j.heliyon.2020.e03946

Gaston, R. T., Ramroop, S., & Habyarimana, F. (2021). Joint modelling of malaria and anaemia in children less than five years of age in Malawi. *Heliyon*, *7*(5). https://doi.org/10.1016/J.HELIYON.2021.E06899

Gidey, B., Nega, D., Abera, A., Abebe, A., Mekasha, S., Tasew, G., Haile, M., Dillu, D., Mehari, D., Assefa, A., Liknew, W., G/Tsadik, A., Mohammed, H., Woldie, E., Getachew, T., Ararso, D., Yenealem, D., Kebede, A., Etana, K., … Abate, E. (2021). Mentorship on malaria microscopy diagnostic service in Ethiopia: baseline competency of microscopists and performance of health facilities. *Malaria Journal*, *20*(1). https://doi.org/10.1186/S12936-021-03655-9

Golwala, Z. M., Shah, H., Gupta, N., Sreenivas, V., & Puliyel, J. M. (2016). Mean platelet volume (MPV), platelet distribution width (PDW), platelet Count and plateletcrit (PCT) as predictors of in-hospital paediatric mortality: A case-control study. *African Health Sciences*, *16*(2), 356–362. https://doi.org/10.4314/ahs.v16i2.3

Hamilton, J. L., & John, S. P. (2013). Evaluation of Fever in Infants and Young Children. *Afp*, *87*(4), 254–260. https://www.aafp.org/pubs/afp/issues/2013/0215/p254.html

Hemingway, J. (2015). Malaria: Fifteen years of interventions. *Nature*, *526*(7572), 198–199. https://doi.org/10.1038/526198A

Henriquez-Camacho, C., & Losa, J. (2014). Biomarkers for sepsis. *BioMed Research International*, *2014*. https://doi.org/10.1155/2014/547818

Herlihy, J. M., D'Acremont, V., Hay Burgess, D. C., & Hamer, D. H. (2016). Diagnosis and Treatment of the Febrile Child. In R. E. Black, R. Laxminarayan, M. Temmerman, & N. Walker (Eds.), *Reproductive, Maternal, Newborn, and Child Health: Disease Control Priorities, Third Edition (Volume 2)*. The International Bank for Reconstruction and Development / The World Bank. http://www.ncbi.nlm.nih.gov/books/NBK361919/

Jagau, H., Behrens, I.-K., Lahme, K., Lorz, G., Köster, R. W., Schneppenheim, R., Obser, T., Brehm, M. A., König, G., Kohler, T. P., Rohde, M., Frank, R., Tegge, W., Fulde, M., Hammerschmidt, S., Steinert, M., & Bergmann, S. (2019). Von Willebrand Factor Mediates Pneumococcal Aggregation and Adhesion in Blood Flow. *Frontiers in Microbiology*, *10*(MAR), 511. https://doi.org/10.3389/fmicb.2019.00511

Jarczak, D., & Nierhaus, A. (2022). Cytokine Storm-Definition, Causes, and Implications. *International Journal of Molecular Sciences*, *23*(19). https://doi.org/10.3390/IJMS231911740

Jimenez, A., Rees-Channer, R. R., Perera, R., Gamboa, D., Chiodini, P. L., González, I. J., Mayor, A., & Ding, X. C. (2017). Analytical sensitivity

of current best-in-class malaria rapid diagnostic tests. *Malaria Journal*, *16*(1). https://doi.org/10.1186/s12936-017-1780-5

Kalumbi, M. H., Katuah, Z. J., Nyirenda, A. E., Katiniche, B., Chinyama, R., Moyo, D., Thugo, S., Mlozen, M., Kamwendo, C., Bonya, E., Nyanda, A. M., Majamanda, J., Taika, W., Chagwa, P., & Linje, L. (n.d.). *Prevalence of Multidrug-Resistant Bacterial Isolates from Chickens, Goats, Cattle and Pigs in Bvumbwe, Malawi*. *234*.

Kaneko, N., Kurata, M., Yamamoto, T., Morikawa, S., & Masumoto, J. (2019). The role of interleukin-1 in general pathology. *Inflammation and Regeneration*, *39*(1), 12. https://doi.org/10.1186/s41232-019-0101-5

Kang, S., Tanaka, T., & Kishimoto, T. (2015). Therapeutic uses of anti-interleukin-6 receptor antibody. *International Immunology*, *27*(1), 21–29. https://doi.org/10.1093/intimm/dxu081

Kapito-Tembo, A., Mathanga, D., Bauleni, A., Nyirenda, O., Pensulo, P., Ali, D., Valim, C., Taylor, T. E., & Laufer, M. K. (2020). Prevalence and Clinical Management of Non-malarial Febrile Illnesses among Outpatients in the Era of Universal Malaria Testing in Malawi. *Am J Trop Med Hyg*, *103*(2), 887–893. https://doi.org/10.4269/ajtmh.18-0800

Kawaguchi, K., Donkor, E., Lal, A., Kelly, M., & Wangdi, K. (2022). Distribution and Risk Factors of Malaria in the Greater Accra Region in Ghana. *International Journal of Environmental Research and Public Health*, *19*(19). https://doi.org/10.3390/IJERPH191912006

Kilpatrick, R., Greenberg, R., Hansen, N. I., Shankaran, S., Carlo, W. A., Cotten, C. M., & Stoll, B. J. (2024). Use and utility of C-reactive protein (CRP) in neonatal early-onset sepsis: a secondary analysis of a prospective surveillance study. *Journal of Perinatology : Official Journal of the California Perinatal Association*. https://doi.org/10.1038/S41372-024-02064-5

Kotepui, M., Kwankaew, P., Mahittikorn, A., Kotepui, K. U., Masangkay, F. R., Wattanapisit, A., & Wilairatana, P. (2024). A systematic review and meta-analysis of blood level of MCP-1/CCL-2 in severe and uncomplicated malaria. *Scientific Reports*, *14*(1), 28738. https://doi.org/10.1038/s41598-024-80201-y

Kularatne, D., Chulasiri, P., Dharmapala, A., & Kularatne, S. (2023). Plasmodium ovale infection in Sri Lanka: distant exposure and incidental detection of hyperparasitemia: a case report. *Journal of Medical Case Reports*, *17*(1). https://doi.org/10.1186/S13256-023-04226-Z

Kumar, R., Ng, S., & Engwerda, C. (2019). The Role of IL-10 in Malaria: A Double Edged Sword. *Front Immunol*, *10*, 229. https://doi.org/10.3389/fimmu.2019.00229

Laurent, P., Lapoirie, J., Leleu, D., Levionnois, E., Grenier, C., Jurado-Mestre, B., Lazaro, E., Duffau, P., Richez, C., Seneschal, J., Pellegrin, J. L., Constans, J., Schaeverbeke, T., Douchet, I., Duluc, D., Pradeu, T., Chizzolini, C., Blanco, P., Truchetet, M. E., & Contin-Bordes, C. (2022). Interleukin-1β-Activated Microvascular Endothelial Cells

Promote DC-SIGN-Positive Alternatively Activated Macrophages as a Mechanism of Skin Fibrosis in Systemic Sclerosis. *Arthritis & Rheumatology (Hoboken, N.J.)*, *74*(6), 1013–1026. https://doi.org/10.1002/ART.42061

Leal-Santos, F. A., Silva, S. B., Crepaldi, N. P., Nery, A. F., Martin, T. O., Alves-Junior, E. R., & Fontes, C. J. (2013). Altered platelet indices as potential markers of severe and complicated malaria caused by Plasmodium vivax: A cross-sectional descriptive study. *Malaria Journal*, *12*(1). https://doi.org/10.1186/1475-2875-12-462

Lenting, P. J., Christophe, O. D., & Denis, C. V. (2015). von Willebrand factor biosynthesis, secretion, and clearance: connecting the far ends. *Blood*, *125*(13), 2019–2028. https://doi.org/10.1182/BLOOD-2014-06-528406

Li, J., Song, J., Kang, L., Huang, L., Zhou, S., Hu, L., Zheng, J., Li, C., Zhang, X., He, X., Zhao, D., Bu, Z., & Weng, C. (2021). pMGF505-7R determines pathogenicity of African swine fever virus infection by inhibiting IL-1β and type I IFN production. *PLoS Pathog*, *17*(7), e1009733. https://doi.org/10.1371/journal.ppat.1009733

Long, S. S. (2016). Diagnosis and management of undifferentiated fever in children. *J Infect*, *72 Suppl*, S68–76. https://doi.org/10.1016/j.jinf.2016.04.025

Lovegrove, F. E., Tangpukdee, N., Opoka, R. O., Lafferty, E. I., Rajwans, N., Hawkes, M., Krudsood, S., Looareesuwan, S., John, C. C., Liles, W. C., & Kain, K. C. (2009). Serum angiopoietin-1 and -2 levels discriminate

cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLoS ONE*, *4*(3). https://doi.org/10.1371/journal.pone.0004912

Lubell, Y., Blacksell, S. D., Dunachie, S., Tanganuchitcharnchai, A., Althaus, T., Watthanaworawit, W., Paris, D. H., Mayxay, M., Peto, T. J., Dondorp, A. M., White, N. J., Day, N. P. J., Nosten, F., Newton, P. N., & Turner, P. (2015). Performance of C-reactive protein and procalcitonin to distinguish viral from bacterial and malarial causes of fever in Southeast Asia. *BMC Infectious Diseases*, *15*(1). https://doi.org/10.1186/s12879-015-1272-6

Lyke, K. E., Burges, R., Cissoko, Y., Sangare, L., Dao, M., Diarra, I., Kone, A., Harley, R., Plowe, C. V, Doumbo, O. K., & Sztein, M. B. (2004). Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. *Infect Immun*, *72*(10), 5630–5637. https://doi.org/10.1128/IAI.72.10.5630-5637.2004

Ma, S., Zhao, M. L., Wang, K., Yue, Y. F., Sun, R. Q., Zhang, R. M., Wang, S. F., Sun, G., Xie, H. Q., Yu, Y., Wang, L., Zhang, W. P., Wang, S. J., Zhu, H. C., & Wang, L. (2020). Association of Ang-2, vWF, and EVLWI with risk of mortality in sepsis patients with concomitant ARDS: A retrospective study. *Journal of the Formosan Medical Association = Taiwan Yi Zhi*, *119*(5), 950–956. https://doi.org/10.1016/J.JFMA.2019.11.005

Madut, D. B., Rubach, M. P., Bonnewell, J. P., Cutting, E. R., Carugati, M., Kalengo, N., Maze, M. J., Morrissey, A. B., Mmbaga, B. T., Lwezaula, B. F., Kinabo, G., Mbwasi, R., Kilonzo, K. G., Maro, V. P., & Crump, J. A. (2021). Trends in fever case management for febrile inpatients in a low malaria incidence setting of Tanzania. *Tropical Medicine & International Health : TM & IH*, *26*(12), 1668–1676. https://doi.org/10.1111/TMI.13683

Mahanta, A., Kar, S. K., Kakati, S., & Baruah, S. (2015). Heightened inflammation in severe malaria is associated with decreased IL-10 expression levels and neutrophils. *Innate Immun*, *21*(5), 546–552. https://doi.org/10.1177/1753425914561277

Mahende, C., Ngasala, B., Lusingu, J., Yong, T.-S., Lushino, P., Lemnge, M., Mmbando, B., & Premji, Z. (2016). Performance of rapid diagnostic test, blood-film microscopy and PCR for the diagnosis of malaria infection among febrile children from Korogwe District, Tanzania. *Malaria Journal*, *15*(1), 391. https://doi.org/10.1186/s12936-016-1450-z

Mahittikorn, A., Kwankaew, P., Rattaprasert, P., Kotepui, K. U., Masangkay, F. R., & Kotepui, M. (2022). Elevation of serum interleukin-1β levels as a potential indicator for malarial infection and severe malaria: a meta-analysis. *Malaria Journal*, *21*(1). https://doi.org/10.1186/s12936-022-04325-0

Majidpoor, J., & Mortezaee, K. (2022). Interleukin-6 in SARS-CoV-2 induced disease: Interactions and therapeutic applications. *Biomedicine and Pharmacotherapy*, *145*. https://doi.org/10.1016/j.biopha.2021.112419

Manca, C., Hill, C., Hujer, A. M., Patel, R., Evans, S. R., Bonomo, R. A., & Kreiswirth, B. N. (2017). Leading antibacterial laboratory research by integrating conventional and innovative approaches: The Laboratory Center of the Antibacterial Resistance Leadership Group. *Clinical Infectious Diseases*, *64*, S13–S17. https://doi.org/10.1093/cid/ciw826

Mandala, W., Munyenyembe, A., Sulani, I., Soko, M., Mallewa, J., & Hiestand, J. (2022). Acute Malaria in Malawian Children and Adults is Characterized by Thrombocytopenia That Normalizes in Convalescence. *Journal of Blood Medicine*, *13*, 485–494. https://doi.org/10.2147/JBM.S376476

Masse, E., & Hantson, P. (2014). Plasmodium falciparum Malaria Complicated by Symmetrical Peripheral Gangrene, Bowel Ischemia, Repeated Candidemia, and Bacteraemia. *Case Reports in Medicine*, *2014*. https://doi.org/10.1155/2014/696725

Mathur, N. B., & Behera, B. (2019). Blood procalcitonin levels and duration of antibiotics in neonatal sepsis. *Journal of Tropical Pediatrics*, *65*(4), 315–320. https://doi.org/10.1093/tropej/fmy053

Mawson, A. R. (2013). The pathogenesis of malaria: a new perspective. *Pathog Glob Health*, *107*(3), 122–129. https://doi.org/10.1179/2047773213Y.0000000084

McComb, S., Thiriot, A., Akache, B., Krishnan, L., & Stark, F. (2019). Introduction to the Immune System. *Methods Mol Biol*, *2024*, 1–24. https://doi.org/10.1007/978-1-4939-9597-4_1

McElvaney, O. J., Curley, G. F., Rose-John, S., & McElvaney, N. G. (2021). Interleukin-6: obstacles to targeting a complex cytokine in critical illness. *The Lancet. Respiratory Medicine*, *9*(6), 643–654. https://doi.org/10.1016/S2213-2600(21)00103-X

McMorran, B. J., Marshall, V. M., De Graaf, C., Drysdale, K. E., Shabbar, M., Smyth, G. K., Corbin, J. E., Alexander, W. S., & Foote, S. J. (2009). Platelets kill intraerythrocytic malarial parasites and mediate survival to infection. *Science*, *323*(5915), 797–800. https://doi.org/10.1126/science.1166296

Mhalu, F. S. (2005). Burden of diseases in poor resource countries: meeting the challenges of combating HIV/AIDS, tuberculosis and malaria. *Tanzania Health Research Bulletin*, *7*(3), 179–184. https://doi.org/10.4314/THRB.V7I3.14257

Millar, S. B., & Cox-Singh, J. (2015). Human infections with Plasmodium knowlesi-zoonotic malaria. *Clinical Microbiology and Infection*, *21*(7), 640–648. https://doi.org/10.1016/j.cmi.2015.03.017

Minderhoud, T. C., Azijli, K., & Nanayakkara, P. W. B. (2020). Sepsis. *The Lancet*, *396*(10265), 1804. https://doi.org/10.1016/S0140-6736(20)32401-6

Morrell, C. N. (2014). Understanding platelets in malaria infection. *Current Opinion in Hematology*, *21*(5), 445–449. https://doi.org/10.1097/MOH.0000000000000073

Mota, C. M. D., & Madden, C. J. (2022). Neural circuits mediating circulating interleukin-1β-evoked fever in the absence of prostaglandin E2 production. *Brain, Behavior, and Immunity*, *103*, 109–121. https://doi.org/10.1016/J.BBI.2022.04.008

Mouatcho, J. C., & Dean Goldring, J. P. (2013). Malaria rapid diagnostic tests: challenges and prospects. *Journal of Medical Microbiology*, *62*(Pt 10), 1491–1505. https://doi.org/10.1099/JMM.0.052506-0

Munyenyembe, A. U., Gausi, K., Nyirenda, T. S., Hiestand, J., Mallewa, J., & Mandala, W. L. (2018). HIV infection has a profound effect on hematological factors but not on electrolyte profile of Malawian adults presenting with uncomplicated malaria and severe malaria. *Journal of Blood Medicine*, *9*, 153–162. https://doi.org/10.2147/JBM.S172869

Nega, D., Abebe, A., Abera, A., Gidey, B., Gtsadik, A., & Tasew, G. (2020). Comprehensive competency assessment of malaria microscopists and laboratory diagnostic service capacity in districts stratified for malaria elimination in Ethiopia. *PLoS ONE*, *15*(6). https://doi.org/10.1371/journal.pone.0235151

Netea, M. G., Domínguez-Andrés, J., Barreiro, L. B., Chavakis, T., Divangahi, M., Fuchs, E., Joosten, L. A. B., van der Meer, J. W. M., Mhlanga, M. M., Mulder, W. J. M., Riksen, N. P., Schlitzer, A., Schultze, J. L., Stabell

Benn, C., Sun, J. C., Xavier, R. J., & Latz, E. (2020). Defining trained immunity and its role in health and disease. *Nat Rev Immunol*, *20*(6), 375–388. https://doi.org/10.1038/s41577-020-0285-6

Ngwengi, N. Y., Fon, P. N., & Mbanya, D. (2020). Distribution of haemoglobin genotypes, knowledge, attitude and practices towards sickle cell disease among unmarried youths in the buea health district, cameroon. *Pan African Medical Journal*, *37*, 1–9. https://doi.org/10.11604/pamj.2020.37.109.17864

Ngwengi, Y., Ngaba, G. P., Nida, M., & Enyama, D. (2023). Evaluation of CRP as a marker for bacterial infection and malaria in febrile children at the Douala Gyneco-Obstetric and Pediatric Hospital. *PloS One*, *18*(7). https://doi.org/10.1371/JOURNAL.PONE.0289012

Nijman, R. G., Moll, H. A., Smit, F. J., Gervaix, A., Weerkamp, F., Vergouwe, Y., De Rijke, Y. B., & Oostenbrink, R. (2014). C-reactive protein, procalcitonin and the lab-score for detecting serious bacterial infections in febrile children at the emergency department: a prospective observational study. *The Pediatric Infectious Disease Journal*, *33*(11), e273–e279. https://doi.org/10.1097/INF.0000000000000466

Nkansah, C., Osei-Boakye, F., Abbam, G., Appiah, S. K., Daud, S., Boakye, B., Abdulai, S., Ahmed, M., Antwi, T. B., Boateng, B., Libatin, M. P., Mensah, A. S., Missah, M. K., Duneeh, R. V., Haruna, A., Adda, S., Abdul-Rauf, P. G., Ofori, Z. A., Fosu, G. B., … Chukwurah, E. F. (2024). Pro- and anti-inflammatory cytokines mediate the progression of severe anemia in malaria-infected children: A prospective study.

Immunity, Inflammation and Disease, *12*(9). https://doi.org/10.1002/iid3.70013

Noh, S., Kim, J., Kim, G., Park, C., Jang, H., Lee, M., & Lee, T. (2021). Recent Advances in CRP Biosensor Based on Electrical, Electrochemical and Optical Methods. *Sensors (Basel, Switzerland)*, *21*(9). https://doi.org/10.3390/S21093024

Nyirenda, T. S., Molyneux, M. E., Kenefeck, R., Walker, L. S. K., MacLennan, C. A., Heyderman, R. S., & Mandala, W. L. (2015). T-regulatory cells and inflammatory and inhibitory cytokines in Malawian children residing in an area of high and an area of low malaria transmission during acute uncomplicated malaria and in convalescence. *Journal of the Pediatric Infectious Diseases Society*, *4*(3), 232–241. https://doi.org/10.1093/jpids/piu140

Obeng-Aboagye, E., Frimpong, A., Amponsah, J. A., Danso, S. E., Owusu, E. D. A., & Ofori, M. F. (2023). Inflammatory cytokines as potential biomarkers for early diagnosis of severe malaria in children in Ghana. *Malaria Journal*, *22*(1). https://doi.org/10.1186/S12936-023-04652-W

Okomo, U., Akpalu, E. N. K., Le Doare, K., Roca, A., Cousens, S., Jarde, A., Sharland, M., Kampmann, B., & Lawn, J. E. (2019). Aetiology of invasive bacterial infection and antimicrobial resistance in neonates in sub-Saharan Africa: a systematic review and meta-analysis in line with the STROBE-NI reporting guidelines. *The Lancet. Infectious Diseases*, *19*(11), 1219–1234. https://doi.org/10.1016/S1473-3099(19)30414-1

Okoro, C. I., Ihenetu, F. C., Dunga, K. E., Achigbu, K., Obasi, C. C., Odinaka, K. K., & Anikwo, E. S. (2020). Fever Cases Associated with Plasmodium falciparum Malaria Infection among Children Attending a Tertiary Health Facility in Imo State, Nigeria. *International Journal of TROPICAL DISEASE & Health*, *41*(5), 7–14. https://doi.org/10.9734/ijtdh/2020/v41i530274

Oluboyo, A. O., Chukwu, S. I., Oluboyo, B. O., & Odewusi, O. O. (2020). Evaluation of Angiopoietins 1 and 2 in Malaria-Infested Children. *Journal of Environmental and Public Health*, *2020*. https://doi.org/10.1155/2020/2169763

Ong'echa, J. M., Davenport, G. C., Vulule, J. M., Hittner, J. B., & Perkins, D. J. (2011). Identification of inflammatory biomarkers for pediatric malarial: Anemia severity using novel statistical methods. *Infection and Immunity*, *79*(11), 4674–4680. https://doi.org/10.1128/IAI.05161-11

Orimadegun, A. E., Dada-Adegbola, H. O., Michael, O. S., Adepoju, A. A., Funwei, R. E., Olusola, F. I., Ajayi, I. O. O., Ogunkunle, O. O., Ademowo, O. G., Jegede, A. S., Baba, E., Hamade, P., Webster, J., Chandroman, D., & Falade, C. O. (2022). Non-Malaria Causes of Fever among under-5 Children with Negative Results for Malaria Rapid Diagnostic Test in South-Western Nigeria. *Journal of Tropical Pediatrics*, *68*(4). https://doi.org/10.1093/TROPEJ/FMAC061

Osei-Kwakye, K., Asante, K. P., Mahama, E., Apanga, S., Owusu, R., Kwara, E., Adjei, G., Abokyi, L., Yeetey, E., Dosoo, D. K., Punguyire, D., & Owusu-Agyei, S. (2013). The benefits or otherwise of managing malaria

cases with or without laboratory diagnosis: the experience in a district hospital in Ghana. *PloS One*, *8*(3). https://doi.org/10.1371/JOURNAL.PONE.0058107

Pathak, A., & Agrawal, A. (2019). Evolution of C-Reactive Protein. *Frontiers in Immunology*, *10*(APR). https://doi.org/10.3389/FIMMU.2019.00943

Penda, C. I., ÉpéeEboumbou, P., Ngondi, G., Hzounda Fokou, J. B., Pfoum, C. V., Mbono Betoko, R., Eposse, C., Endale, L. M., Bebey, F. S., & Moukoko, C. E. E. (2023). Etiology and clinical characteristics of pediatric acute fever among hospitalized children in an endemic malaria transmission area of Cameroon in Central Africa. *PLoS ONE*, *18*(1 January). https://doi.org/10.1371/journal.pone.0278407

Phillips, M. A., Burrows, J. N., Manyando, C., van Huijsduijnen, R. H., Van Voorhis, W. C., & Wells, T. N. C. (2017). Malaria. *Nat Rev Dis Primers*, *3*, 17050. https://doi.org/10.1038/nrdp.2017.50

Plucinski, M. M., Candrinho, B., Dimene, M., Smith, T., Thwing, J., Colborn, J., Rogier, E., & Zulliger, R. (2020). Estimation of Malaria-Attributable Fever in Malaria Test-Positive Febrile Outpatients in Three Provinces of Mozambique, 2018. *The American Journal of Tropical Medicine and Hygiene*, *102*(1), 151–155. https://doi.org/10.4269/AJTMH.19-0537

Pokharel, S., Shrestha, P., & Adhikari, B. (2020). Antimicrobial use in food animals and human health: time to implement 'One Health' approach. *Antimicrobial Resistance and Infection Control*, *9*(1). https://doi.org/10.1186/S13756-020-00847-X

Popa, G. L., & Popa, M. I. (2021). Recent Advances in Understanding the Inflammatory Response in Malaria: A Review of the Dual Role of Cytokines. *Journal of Immunology Research*, *2021*. https://doi.org/10.1155/2021/7785180

Post, A., Kaboré, B., Berendsen, M., Diallo, S., Traore, O., Arts, R. J. W., Netea, M. G., Joosten, L. A. B., Tinto, H., Jacobs, J., de Mast, Q., & van der Ven, A. (2021). Altered Ex-Vivo Cytokine Responses in Children With Asymptomatic Plasmodium falciparum Infection in Burkina Faso: An Additional Argument to Treat Asymptomatic Malaria? *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.614817

Póvoa, P., & Coelho, L. (2021). Which Biomarkers Can Be Used as Diagnostic Tools for Infection in Suspected Sepsis? *Seminars in Respiratory and Critical Care Medicine*, *42*(5), 662–671. https://doi.org/10.1055/s-0041-1735148

Póvoa, P., Coelho, L., & Bos, L. D. J. (2020). New biomarkers for respiratory infections. *Current Opinion in Pulmonary Medicine*, *26*(3), 232–240. https://doi.org/10.1097/MCP.0000000000000669

Pratt, A., & Attia, M. W. (2007). Duration of fever and markers of serious bacterial infection inyoung febrile children. *Pediatrics International*, *49*(1), 31–35. https://doi.org/10.1111/j.1442-200X.2007.02316.x

Průcha, M., Zazula, R., & Russwurm, S. (2018). Sepsis Diagnostics in the Era of "Omics" Technologies. *Prague Medical Report*, *119*(1), 9–29. https://doi.org/10.14712/23362936.2018.2

Qiao, J., & Fu, Y.-X. (2020). Cytokines that target immune killer cells against tumors. *Cell Mol Immunol*, *17*(7), 722–727. https://doi.org/10.1038/s41423-020-0481-0

Qiao, J., Liu, Z., Dong, C., Luan, Y., Zhang, A., Moore, C., Fu, K., Peng, J., Wang, Y., Ren, Z., Han, C., Xu, T., & Fu, Y. X. (2019). Targeting Tumors with IL-10 Prevents Dendritic Cell-Mediated CD8+ T Cell Apoptosis. *Cancer Cell*, *35*(6), 901-915.e4. https://doi.org/10.1016/J.CCELL.2019.05.005

Requena, P., Barrios, D., Robinson, L. J., Samol, P., Umbers, A. J., Wangnapi, R., Ome-Kaius, M., Rosanas-Urgell, A., Mayor, A., López, M., de Lazzari, E., Arévalo-Herrera, M., Fernández-Becerra, C., del Portillo, H., Chitnis, C. E., Siba, P. M., Rogerson, S., Mueller, I., Bardají, A., … Dobaño, C. (2015). Proinflammatory responses and higher IL-10 production by T cells correlate with protection against malaria during pregnancy and delivery outcomes. *J Immunol*, *194*(7), 3275–3285. https://doi.org/10.4049/jimmunol.1401038

Rizo-Téllez, S. A., Sekheri, M., & Filep, J. G. (2023). C-reactive protein: a target for therapy to reduce inflammation. *Front Immunol*, *14*, 1237729. https://doi.org/10.3389/fimmu.2023.1237729

Rose-John, S. (2018). Interleukin-6 Family Cytokines. *Cold Spring Harbor Perspectives in Biology*, *10*(2). https://doi.org/10.1101/CSHPERSPECT.A028415

Rudd, K. E., Johnson, S. C., Agesa, K. M., Shackelford, K. A., Tsoi, D., Kievlan, D. R., Colombara, D. V., Ikuta, K. S., Kissoon, N., Finfer, S., Fleischmann-Struzek, C., Machado, F. R., Reinhart, K. K., Rowan, K., Seymour, C. W., Watson, R. S., West, T. E., Marinho, F., Hay, S. I., … Naghavi, M. (2020). Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the Global Burden of Disease Study. *Lancet (London, England)*, *395*(10219), 200–211. https://doi.org/10.1016/S0140-6736(19)32989-7

Saraiva, M., Vieira, P., & O'Garra, A. (2020). Biology and therapeutic potential of interleukin-10. *J Exp Med*, *217*(1), e20190418. https://doi.org/10.1084/jem.20190418

Sarfo, J. O., Amoadu, M., Gyan, T. B., Osman, A. G., Kordorwu, P. Y., Adams, A. K., Asiedu, I., Ansah, E. W., Amponsah-Manu, F., & Ofosu-Appiah, P. (2023). Acute lower respiratory infections among children under five in Sub-Saharan Africa: a scoping review of prevalence and risk factors. *BMC Pediatrics*, *23*(1). https://doi.org/10.1186/s12887-023-04033-x

Sarfo, J. O., Amoadu, M., Kordorwu, P. Y., Adams, A. K., Gyan, T. B., Osman, A. G., Asiedu, I., & Ansah, E. W. (2023). Malaria amongst children under five in sub-Saharan Africa: a scoping review of prevalence, risk factors and preventive interventions. *European Journal of Medical Research*, *28*(1). https://doi.org/10.1186/S40001-023-01046-1

Seidizadeh, O., Eikenboom, J. C. J., Denis, C. V., Flood, V. H., James, P., Lenting, P. J., Baronciani, L., O'Donnell, J. S., Lillicrap, D., &

Peyvandi, F. (2024). von Willebrand disease. *Nature Reviews Disease Primers*, *10*(1). https://doi.org/10.1038/s41572-024-00536-8

Semakula, H. M., Liang, S., Mukwaya, P. I., Mugagga, F., Swahn, M., Nseka, D., Wasswa, H., & Kayima, P. (2023). Determinants of malaria infections among children in refugee settlements in Uganda during 2018-2019. *Infectious Diseases of Poverty*, *12*(1). https://doi.org/10.1186/S40249-023-01090-3

Shahbodaghi, S. D., & Rathjen, N. A. (2022). Malaria: Prevention, Diagnosis, and Treatment. *American Family Physician*, *106*(3), 270–278.

Shukla, S. D., Shastri, M. D., Vanka, S. K., Jha, N. K., Dureja, H., Gupta, G., Chellappan, D. K., Oliver, B. G., Dua, K., & Walters, E. H. (2022). Correction to: Targeting intercellular adhesion molecule-1 (ICAM-1) to reduce rhinovirus-induced acute exacerbations in chronic respiratory diseases (Inflammopharmacology, (2022), 30, 3, (725-735), 10.1007/s10787-022-00968-2). *Inflammopharmacology*, *30*(4), 1477. https://doi.org/10.1007/s10787-022-01018-7

Silva, A. C., Costa, C. P., Almeida, H., Moreira, J. N., & Lobo, J. M. S. (2019). Hormones, blood products, and therapeutic enzymes. *Advances in Biochemical Engineering/Biotechnology*, *171*. https://doi.org/10.1007/10_2019_111

Singh, B., & Daneshvar, C. (2013). Human infections and detection of Plasmodium knowlesi. *Clinical Microbiology Reviews*, *26*(2), 165–184. https://doi.org/10.1128/CMR.00079-12

Sornsenee, P., Wilairatana, P., Kotepui, K. U., Masangkay, F. R., Romyasamit, C., & Kotepui, M. (2023). Relation between Increased IL-10 Levels and Malaria Severity: A Systematic Review and Meta-Analysis. *Tropical Medicine and Infectious Disease*, *8*(1). https://doi.org/10.3390/TROPICALMED8010035

Sridharan, P., & Chamberlain, R. S. (2013). The efficacy of procalcitonin as a biomarker in the management of sepsis: Slaying dragons or tilting at windmills? *Surgical Infections*, *14*(6), 489–511. https://doi.org/10.1089/sur.2012.028

Steinert, M., Ramming, I., & Bergmann, S. (2020). Impact of Von Willebrand Factor on Bacterial Pathogenesis. *Frontiers in Medicine*, *7*. https://doi.org/10.3389/FMED.2020.00543

Stockschlaeder, M., Schneppenheim, R., & Budde, U. (2014). Update on von Willebrand factor multimers: focus on high-molecular-weight multimers and their role in hemostasis. *Blood Coagul Fibrinolysis*, *25*(3), 206–216. https://doi.org/10.1097/MBC.0000000000000065

Stoma, I., Karpov, I., Uss, A., Rummo, O., Milanovich, N., & Iskrov, I. (2017). Diagnostic value of sepsis biomarkers in hematopoietic stem cell transplant recipients in a condition of high prevalence of gram-negative pathogens. *Hematology/ Oncology and Stem Cell Therapy*, *10*(1), 15–21. https://doi.org/10.1016/j.hemonc.2016.09.002

Suurbaar, J., Moussiliou, A., Tahar, R., Olsen, R. W., Adams, Y., Dalgaard, N., Baafour, E. K., Adukpo, S., Hviid, L., Kusi, K. A., Alao, J., Ofori, M.

F., Ndam, N. T., & Jensen, A. R. (2022). ICAM-1-binding Plasmodium falciparum erythrocyte membrane protein 1 variants elicits opsonic-phagocytosis IgG responses in Beninese children. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-16305-0

Tanaka, T., & Kishimoto, T. (2012). Immunotherapeutic implication of IL-6 blockade. *Immunotherapy*, *4*(1), 87–105. https://doi.org/10.2217/imt.11.147

Tanaka, T., Narazaki, M., & Kishimoto, T. (2014). IL-6 in inflammation, immunity, and disease. *Cold Spring Harbor Perspectives in Biology*, *6*(10). https://doi.org/10.1101/CSHPERSPECT.A016295

Tang, W., Zhang, Y., Cui, S., & Yi, F. (2022). The Growth Factors: Potential Biomarkers and Therapeutic Targets in Kidney Diseases. *Kidney Diseases (Basel, Switzerland)*, *8*(5), 368–380. https://doi.org/10.1159/000526208

The 2023 WHO World malaria report - The Lancet Microbe. (n.d.). Retrieved December 18, 2024, from https://www.thelancet.com/journals/lanmic/article/PIIS2666-5247%2824%2900016-8/fulltext

Thomson, A., Khogali, M., De Smet, M., Reid, T., Mukhtar, A., Peterson, S., & Von Schreeb, J. (2011). Low referral completion of rapid diagnostic test-negative patients in community-based treatment of malaria in Sierra Leone. *Malaria Journal*, *10*. https://doi.org/10.1186/1475-2875-10-94

Thurman, A. R., Kimble, T., Herold, B., Mesquita, P. M. M., Fichorova, R. N., Dawood, H. Y., Fashemi, T., Chandra, N., Rabe, L., Cunningham, T. D., Anderson, S., Schwartz, J., & Doncel, G. (2015). Bacterial Vaginosis and Subclinical Markers of Genital Tract Inflammation and Mucosal Immunity. *AIDS Research and Human Retroviruses*, *31*(11), 1139–1152. https://doi.org/10.1089/AID.2015.0006

Tsalik, E. L., Petzold, E., Kreiswirth, B. N., Bonomo, R. A., Banerjee, R., Lautenbach, E., Evans, S. R., Hanson, K. E., Klausner, J. D., Patel, R., Caliendo, A., Chiu, C., Humphries, R., Miller, M., & Woods, C. (2017). Advancing Diagnostics to Address Antibacterial Resistance: The Diagnostics and Devices Committee of the Antibacterial Resistance Leadership Group. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *64*(suppl_1), S41–S47. https://doi.org/10.1093/CID/CIW831

Tuyen, T. T., Viet, N. T., Hang, N. T., Giang, N. T., Anh, D. D., Anh, D. T., Hung, H. V., Quyet, D., Toan, N. L., Cam, T. D., & Van Tong, H. (2020). Proinflammatory Cytokines Are Modulated in Vietnamese Patients with Dengue Fever. *Viral Immunology*, *33*(7), 514–520. https://doi.org/10.1089/vim.2020.0023

Valim, C., Ahmad, R., Lanaspa, M., Tan, Y., Acácio, S., Gillette, M. A., Almendinger, K. D., Milner, D. A., Madrid, L., Pellé, K., Harezlak, J., Silterra, J., Alonso, P. L., Carr, S. A., Mesirov, J. P., Wirth, D. F., Wiegand, R. C., & Bassat, Q. (2016). Responses to Bacteria, Virus, and Malaria Distinguish the Etiology of Pediatric Clinical Pneumonia. *Am J*

Respir Crit Care Med, *193*(4), 448–459. https://doi.org/10.1164/rccm.201506-1100OC

van Engelen, T. S. R., Wiersinga, W. J., Scicluna, B. P., & van der Poll, T. (2018). Biomarkers in Sepsis. *Critical Care Clinics*, *34*(1), 139–152. https://doi.org/10.1016/J.CCC.2017.08.010

van Seventer, J. M., & Hochberg, N. S. (2017). Principles of Infectious Diseases: Transmission, Diagnosis, Prevention, and Control. *International Encyclopedia of Public Health*, 22–39. https://doi.org/10.1016/B978-0-12-803678-5.00516-6

Varo, R., Chaccour, C., & Bassat, Q. (2020). Update on malaria. *Medicina Clinica*, *155*(9), 395–402. https://doi.org/10.1016/J.MEDCLI.2020.05.010

Venkatesan, P. (2024). The 2023 WHO World malaria report. *The Lancet Microbe*, *5*(3), e214. https://doi.org/10.1016/s2666-5247(24)00016-8

Vincent, J. L. (2020). Highlighting the huge global burden of sepsis. *Anaesthesia Critical Care and Pain Medicine*, *39*(2), 171–172. https://doi.org/10.1016/j.accpm.2020.03.004

Vujevic, M., Benzon, B., & Markic, J. (2017). New prediction model for diagnosis of bacterial infection in febrile infants younger than 90 days. *Turkish Journal of Pediatrics*, *59*(3), 261–268. https://doi.org/10.24953/turkjped.2017.03.005

Wainaina, M., Silva, D. A. V. da, Dohoo, I., Mayer-Scholl, A., Roesel, K., Hofreuter, D., Roesler, U., Lindahl, J., Bett, B., & Dahouk, S. Al. (2022). A systematic review and meta-analysis of the aetiological agents of non-malarial febrile illnesses in Africa. *PLOS Neglected Tropical Diseases*, *16*(1), e0010144. https://doi.org/10.1371/journal.pntd.0010144

Walker, I. S., & Rogerson, S. J. (2023). Pathogenicity and virulence of malaria: Sticky problems and tricky solutions. *Virulence*, *14*(1). https://doi.org/10.1080/21505594.2022.2150456

Wambani, J., & Okoth, P. (2022). Impact of Malaria Diagnostic Technologies on the Disease Burden in the Sub-Saharan Africa. *Journal of Tropical Medicine*, *2022*. https://doi.org/10.1155/2022/7324281

Wang, J. W., Groeneveld, D. J., Cosemans, G., Dirven, R. J., Valentijn, K. M., Voorberg, J., Reitsma, P. H., & Eikenboom, J. J. (2012). Biogenesis of Weibel-Palade bodies in von Willebrand's disease variants with impaired von Willebrand factor intrachain or interchain disulfide bond formation. *Haematologica*, *97*(6), 859–866. https://doi.org/10.3324/haematol.2011.057216

Wang, S., Xu, K., & Wang, G. (2024). Delayed diagnosis of persistent Q fever: a case series from China. *BMC Infectious Diseases*, *24*(1). https://doi.org/10.1186/s12879-024-09484-w

Wassmer, S. C., Taylor, T. E., Rathod, P. K., Mishra, S. K., Mohanty, S., Arevalo-Herrera, M., Duraisingh, M. T., & Smith, J. D. (2015).

Investigating the pathogenesis of severe malaria: A multidisciplinary and cross-geographical approach. *American Journal of Tropical Medicine and Hygiene*, *93*, 42–56. https://doi.org/10.4269/AJTMH.14-0841

Weghorst, A. A. H., Bonvanie, I. J., Holtman, G. A., de Boer, M. R., & Berger, M. Y. (2022). Course of uncomplicated acute gastroenteritis in children presenting to out-of-hours primary care. *BMC Primary Care*, *23*(1). https://doi.org/10.1186/s12875-022-01739-2

Weitkamp, J. H. (2021). The role of biomarkers in suspected neonatal sepsis. *Clinical Infectious Diseases*, *73*(2), E391–E393. https://doi.org/10.1093/cid/ciaa869

Whitburn, S., Costelloe, C., Montgomery, A. A., Redmond, N. M., Fletcher, M., Peters, T. J., & Hay, A. D. (2011). The frequency distribution of presenting symptoms in children aged six months to six years to primary care. *Primary Health Care Research & Development*, *12*(2), 123–134. https://doi.org/10.1017/S146342361000040X

Wilairatana, P., Mala, W., Milanez, G. D. J., Masangkay, F. R., Kotepui, K. U., & Kotepui, M. (2022). Increased interleukin-6 levels associated with malaria infection and disease severity: a systematic review and meta-analysis. *Scientific Reports*, *12*(1). https://doi.org/10.1038/S41598-022-09848-9

World Health Organization (WHO). (n.d.). Retrieved December 18, 2024, from https://www.who.int/

Xiao, Y., Luo, H., Zhou, B., Dai, X., Huang, J., Duan, L., You, Y., Zhang, W., Zhao, H., Xie, Y., Zhou, Y., Ning, W., Li, T., Liu, S., Zhu, H., Xie, X., Jiang, Y., Wu, S., He, W., & Li, Y. (2017). Comparison of soluble urokinase plasminogen activator receptor, soluble triggering receptor expressed on myeloid cells 1, procalcitonin and C-reactive protein in distinguishing concurrent bacterial infection from idiopathic inflammatory myopathy. *Rheumatology International*, *37*(4), 585–592. https://doi.org/10.1007/s00296-016-3609-x

Yang, S., & Rothman, R. E. (2004). PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings. *Lancet Infect Dis*, *4*(6), 337–348. https://doi.org/10.1016/S1473-3099(04)01044-8

Yazdi, A. S., & Ghoreschi, K. (2016a). The Interleukin-1 Family. *Advances in Experimental Medicine and Biology*, *941*, 21–29. https://doi.org/10.1007/978-94-024-0921-5_2

Yazdi, A. S., & Ghoreschi, K. (2016b). The Interleukin-1 Family. *Adv Exp Med Biol*, *941*, 21–29. https://doi.org/10.1007/978-94-024-0921-5_2

Yu, X., Shang, H., & Jiang, Y. (2020). ICAM-1 in HIV infection and underlying mechanisms. *Cytokine*, *125*. https://doi.org/10.1016/J.CYTO.2019.154830

Zdrodowska, A., Zajkowska, J., Golian, J., Grygorczuk, S., Krupa, W., & Kondrusik, M. (2006). Tropical malaria--danger for persons visiting endemic areas. *Przegląd Lekarski*, *63*(3), 162–165.

Zeukeng, F., Tchinda, V. H. M., Bigoga, J. D., Seumen, C. H. T., Ndzi, E. S., Abonweh, G., Makoge, V., Motsebo, A., & Moyou, R. S. (2014). Co-infections of malaria and geohelminthiasis in two rural communities of Nkassomo and Vian in the Mfou health district, Cameroon. *PLoS Neglected Tropical Diseases*, *8*(10). https://doi.org/10.1371/JOURNAL.PNTD.0003236

Zhai, X., Kong, W. G., Cheng, G. F., Cao, J. F., Dong, F., Han, G. K., Song, Y. L., Qin, C. J., & Xu, Z. (2021). Molecular Characterization and Expression Analysis of Intercellular Adhesion Molecule-1 (ICAM-1) Genes in Rainbow Trout (Oncorhynchus mykiss) in Response to Viral, Bacterial and Parasitic Challenge. *Frontiers in Immunology*, *12*. https://doi.org/10.3389/FIMMU.2021.704224

Zhang, H., Fink, G., & Cohen, J. (2024). Malaria Rapid Tests, Febrile Illness Management, and Child Mortality Across Sub-Saharan African Countries. *JAMA*, *332*(15), 1270–1281. https://doi.org/10.1001/jama.2024.12589

Zhang, L., Zhang, J., He, H., Ling, X., Li, F., Yang, Z., Zhao, J., Li, H., Yang, T., Zhao, S., Shi, K., Guan, X., Zhao, R., & Li, Z. (2022). Increased Cytokine Levels Assist in the Diagnosis of Respiratory Bacterial Infections or Concurrent Bacteremia in Patients With Non-Hodgkin's Lymphoma. *Front Cell Infect Microbiol*, *12*, 860526. https://doi.org/10.3389/fcimb.2022.860526