The Potential of Intermittent Screening and Treatment with Dihydroartemisinin-Piperaquine for the Control of Malaria in Pregnancy in Areas with High Sulphadoxine-Pyrimethamine Resistance

By

Mwayiwawo Morrison Madanitsa

(MBBS)

Thesis submitted in accordance with the requirements of the University of Liverpool and University of Malawi for the degree of Doctor in Philosophy

July 2015

CERTIFICATE OF APPROVAL

The thesis of Mwayiwawo M. Madanitsa is approved by the Thesis Examination Committee:

Prof. Victor Mwapasa

(Chairman, Post Graduate Committee)

Prof. Feiko ter Kuile and Prof. Victor Mwapasa

(Supervisors)

Dr. Dianne J Terlouw and Prof. Kamija Phiri

(Internal Examiners)

Dr. Gertrude Chapotera

(Head of Department)

STATEMENT OF OWN WORK

I, Mwayiwawo Morrison Madanitsa, confirm that the work presented in this thesis is my own, completed under the primary supervision of Professor Feiko O ter Kuile and secondary supervision of Dr Linda Kalilani-Phiri and Professor Victor Mwapasa.

The development of the proposal and protocol of the works presented in this thesis was a shared effort with the primary supervisors. Professor Feiko ter Kuile and Dr Linda Kalilani conceived the concept of the study and acquired the funding. I undertook further revision of the concept and was responsible for the day to day administration of the field work, providing oversight on all requirements for conduct of the trial in compliance with ICH-GCP guidelines.

The development of the statistical analysis plan for the main trial was undertaken with Mr Arthur Kang'ombe, Professor Brian Faragher, Professor Dualao Wang and Professor Feiko ter Kuile. The write up of the main trial chapter was shared with Professor Feiko ter Kuile as a draft for separate manuscript publication. I was solely responsible for the analysis of the results and write-up of all subsequent chapters.

Contributions by the following individuals have been integral to the completion of this work as follows:

- Data management was with the assistance of Mr Alfred Malili and Mr James Smedley (CoM and LSTM respectively). Further assistance with data management and manipulation, was provided by Dr Annemieke van Eijk and Ms Carole Khairallah (LSTM).
- Assistance with statistical analysis for the main trial was provided by Professors Brian Faragher, Dualao Wang, Feiko ter Kuile and Ms Carole Khairallah.
- PCR assays were performed by Dr Steve Taylor and Mr Kyaw-Lay Thwai (Gillings School of Global Public Health, University of North Carolina)

- Malaria thick smear reading and placental histology was undertaken by Mr Humphries Malata and Mr K.E.B Makwakwa from the Tropical Haematology Research Unit and Histopathology division at the College of Medicine laboratories respectively.
- Field work was conducted with the assistance of a team of four research nurses and Dr Doreen Ali (MoH) provided invaluable liaison with the Ministry of Health for conduct of the trial.

Due acknowledgment shall be given in all works to be published in recognition of their invaluable contributions.

Signed: _____

Date: _____

Mwayiwawo M. Madanitsa, July 2015

ACKNOWLEDGEMENTS

The culmination of this work would not have been possible without the invaluable contributions of several individuals, through their technical expertise and moral support.

My heartfelt gratitude goes to Dr Linda Kalilani-Phiri and Professor Victor Mwapasa for their untiring support and inspiration from as early as my second year as an undergraduate medical student. Their support and inspiration through the years have been the source of motivation. Thank you for the inspiration and mentoring through the years.

It was an honor and a privilege to receive the assistance of several outstanding epidemiologists who I am privileged to regard not only as colleagues but also friends. Without mention, Professor Feiko ter Kuile has not only been a great supervisor but also a valued mentor through the course of this work. My thanks go to the members of my academic progress panel, Drs Anja Terlouw and Naor Barzeev, whose insights, moral support and advisement were invaluable.

To the members of MIP secretariat at the Liverpool School of Tropical Medicine, both past and present, their logistical and technical support have had an immeasurable contribution. The fruitful collaborations with other members of the MIP consortium have been invaluable.

To the study team I closely worked with in the conduct of the ISTp-Malawi study, I say thank you for demonstrating rare dedication to your duties and performing the same with the utmost professionalism, without which the successful completion of this work would not have been possible. I extend my appreciations to my fellow doctoral students for the comradeship and encouragement shared.

My greatest appreciations go to my beloved wife and son, who have stood by me through the course of these studies and to my near and extended family for their continuous support and encouragement through the many years.

This work was made possible by the financial support of the European and Developing Countries Clinical Trials Partnership (EDCTP).

DEDICATION

In memory of my late father, Tom Leonard Madanitsa, for always instilling in his children the spirit of academic excellence, diligence and discipline. This achievement would have made him proud

To the memory of my late mother, Christina Madanitsa. Her humility, compassion and faith remain an ever present inspiration and strength. Thank you for teaching us that all things are possible if you can just believe

ABSTRACT

Importance: In Africa most *P. falciparum* malaria infections during pregnancy remain asymptomatic yet are associated with maternal anaemia and low birthweight. WHO recommends intermittent preventive therapy in pregnancy during the second and third trimester with Sulphadoxine-Pyrimethamine (IPTp-SP). However, SP efficacy is threatened by high-level parasite resistance.

Thesis objectives:

The objectives of this thesis were three fold:

Efficacy and safety of ISTp-DP

To evaluate the efficacy and safety of scheduled intermittent screening with malaria rapid diagnostic tests (RDTs) and treatment of RDT-positive women with Dihydroartemisinin-piperaquine (ISTp-DP) as an alternative strategy to IPTp-SP in an area of high malaria transmission and SP resistance.

Effect of asymptomatic malaria infections

To investigate the effect of asymptomatic malaria infections, specifically evaluating the role of asymptomatic infections missed by a malaria rapid diagnostic test (mRDT) on pregnancy and birth outcomes.

Diagnostic sensitivity of HRP2/pLDH RDT for active placental malaria

To determine the sensitivity of an mRDT to detect active malaria infection sequestered in the placenta.

Design, setting, and participants: This was an open-label two-arm individually randomised superiority trial in 3 sites with high SP resistance in Malawi. Between July 2011 and March 2013, 1873 HIV-seronegative women at 16-28 weeks of gestation were recruited (1155 primigravidae and secundigravidae [paucigravidae], 718 multigravidae).

Interventions: IST and IPTp-SP were administered at 3 or 4 scheduled visits in the 2nd and 3rd trimester, 4 to 6 weeks apart. The IPTp-SP arm received SP at each visit. The ISTp-DP arm were screened for malaria at every visit and treated with DP if RDT-positive.

Main outcomes and measures:

Efficacy and safety

The primary outcomes of interest to evaluate the efficacy and safety of ISTp-DP were gravidity dependent. Amongst paucigravidae these were any adverse live birth outcome (composite of small-for-gestation age, low birthweight or preterm birth) whilst amongst multigravidae, any *P. falciparum* infection at delivery was of primary interest. Analysis was by modified intention to treat.

Effect of asymptomatic malaria infections

Outcomes of interest in this analysis were composite adverse live birth outcome, individual adverse live birth outcomes (small for gestational age, preterm birth and low birthweight) and maternal anaemia. Analysis was restricted to only women in the IST arm fulfilling the modified intention to treat criteria.

Diagnostic sensitivity of HRP2/pLDH RDT for active placental malaria

The diagnostic sensitivity of mRDT on peripheral maternal venous blood at delivery for active placental malaria against placental histology as the gold standard was the primary outcome of interest. Analysis included women from both trial arms at delivery.

Results:

Efficacy and safety

The prevalence of adverse birth outcome was similar in both arms: ISTp-DP=29.9%, IPTp-SP=28.8%, Risk-Difference: 1.08%, 95% confidence interval (CI): -3.25 to 5.41; Relative Risk (RR) =1.04 (0.90-1.20), p=0.625, (paucigravidae: RR=1.10 [0.92-1.31], p=0.282; multi-gravidae RR=0.92 [0.71-1.20], p=0.543). The prevalence of malaria at delivery was higher in the ISTp-DP arm (48.7% vs 40.8%): Risk-Difference=7.85 (3.07-12.63); RR=1.19 (1.07-1.33), p=0.007 (paucigravidae: RR=1.16 [1.04-

1.31], p=0.011; multi-gravidae: RR=1.29 [1.02-1.63], p=0.037). Foetal loss was more common with ISTp-DP (2.6% vs 1.3%; RR=2.06 [1.01-4.21], p=0.046) and highest among non DP-recipients (3.1%) in the ISTp-DP arm.

Effect of asymptomatic malaria infections by RDT

46.2% of women had malaria infection by either RDT or PCR at their first antenatal visit. The prevalence of sub-RDT infection was consistently higher in multigravidae than paucigravidae throughout pregnancy. The risk for any placental malaria was higher with asymptomatic missed RDT parasitaemia than never having had any detected parasitaemia at study visits: 38.8% vs 20.4%; RR= 1.90; 95% CI 1.28, 2.82; p=0.002. This was consistent when stratified by gravidity. Missed infections were also associated with higher risk for composite any adverse live birth outcome (26.5% vs 12.8%; RR=2.06; 95%CI 1.23, 3.42; p=0.005) which was driven by the strong association with preterm birth: 19.1% vs 4.1%; RR=4.7; 95% CI 2, 11.1; p<0.001. Asymptomatic patent RDT infections were associated with increased risk for maternal anaemia, placental malaria, composite adverse live birth outcome, preterm birth and low birth weight.

Diagnostic sensitivity of HRP2/pLDH RDT for active placental malaria

Peripheral blood RDTs at delivery were 48% sensitive (95% C.I 39.6, 56.4%) to diagnose active placental malaria infection. This was lower than PCR (64% vs 48%; difference=16.4%; 95% CI 8.4, 24.5%; p<0.001) but higher than LM (48% vs 16%; difference=31.5%; 95% CI 22.4, 40.6%; p<0.001). The sensitivity of RDTs on placental blood to diagnose active placental infection was lower than in peripheral blood: 34% vs 48%; difference=-13.7%; 95% CI -21.1, -6.3%, p<0.001. Peripheral blood RDT had a low diagnostic sensitivity at 70% (95% CI 47.1, 86.8%) in detecting an active placental infection density of above 100parasites/500 RBCs, associated with an increased risk of low birth weight.

Conclusion and relevance: Scheduled screening for malaria parasites with RDTs provided 3 to 4 times during pregnancy as part of focused antenatal care was not superior to IPTp-SP in a setting of

high SP resistance, being associated with higher foetal loss and more malaria at delivery. This may be attributable to the effect of asymptomatic parasitaemia that are left to persists in the peripheral blood without treatment due to low rate of detection of infection by RDTs through subsequent antenatal visits, and/ or the low sensitivity to detect active infection sequestered in the placenta. As such, in areas with high SP resistance, ISTp-DP is not a viable option. There remains an urgent need to identify alternative drugs that can replace SP for antenatal malaria prevention.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS & ACRONYMS

ACT	Artemisinin Combination Therapy
AL	Artemether-Lumefantrine
ANG-	Angiopoietins
AQAS	Amodiaquine-Artesunate
AUC	Area Under the Curve
AVP	Argenine Vasopressin
C-	Complement
CD	Cluster of Differentiation
COX-	Cyclo-oxygenase
CRP	C-Reactive Protein
CSA	Chondroitin Sulfate A
DDT	Dichlorodiphenyltrichloroethane
dhfr	Dihydrofolate reductase
dhps	Dihydropteroate synthase
DNA	Deoxyribonucleic Acid
DOR	Diagnostic Odds Ratio
DP	Dihydroartemisinin-piperaquine
EIR	Entomological Inocculation Rate
ELISA	Enzyme Linked Immunosorbent Assays
FDA	United States Food and Drug Administration
FIND	Foundation for Innovative New Diagnostics
HIF	Hypoxia Inducible Factor

HIV	Human Immnunodeficiency Virus Type 1
HRP2	Plasmodium falciparum Histidine Rich Protein 2
ICAM-1	Intercellular Adhesion Molecule 1
IE	Infected Erythrocytes
IFA	Immunoflorescent Antibody
IFN-γ	Interferon γ
IL -	Interleukin
IPTi	Intermittent Preventive Treatment in infants
IPTp-SP	Intermittent Preventive Treatment with Sulphadoxine-Pyrimethamine
IRS	Indoor Residual Spraying
ISTp-DP	Intermittent Screening and Treatment with Dihydroartemisinin-piperaquine
ITNs	Insecticide Treated Bednets
IUGR	Intrauterine Growth Restriction
LAMP	Loop Mediated Isothermal Amplification
LBW	Low Birth Weight
LLITNs	Long Lasting Insecticide Treated Bednets
LM	Light Microscopy
MIF	Migration Inhibitory Factor
MQ	Mefloquine
NPV	Negative Predictive Value
PCR	Polymerase Chain Reaction
P*f*EMP1	Plasmodium falciparum Erythrocyte Membrane Protein 1
pLDH	Plasmodium lactate dehydrogenase
PPV	Positive Predictive Value

QBC	Quantitative Buffy Coat
RDT	malaria Rapid Diagnostic Test
ROC	Receiver Operating Characteristic
SGA	Small for Gestational Age
SP	Sulphadoxine-Pyrimethamine
SPAZ	Sulphadoxine-Pyrimethamine and Azithromycin
SSTp	Single Screen and Treat in pregnancy
TDR	Special Programme for Research and Training in Tropical Diseases
TGF-β	Transforming Growth Factor β
Th2	Tail Homology 2
TNF α	Tumor Necrosis Factor α
UNICEF	United Nations Children's Fund
VAR2CSA	Variant Surface Antigen 2-Chondroitin Sulfate A
VEGF	VascularEndothelial Growth Factor
WB	World Bank
WHO	World Health Organization

1 CHAPTER ONE: INTRODUCTION

1.1 Research overview

1.1.1 Background

Women bear a greater disease burden in regards to reproductive health than their male counterparts. Pregnancy predisposes women to greater health adversities. In developing countries, infections and nutritional deficiencies pose a constant impediment to the wellbeing of both the woman and unborn child. Malaria in pregnancy remains a major public health challenge in areas of malaria endemicity with significant risks for morbidity and mortality to mother-fetal pairs. By absolute numbers, *Plasmodium vivax* which is endemic in mostly south-east Asia, the Indian subcontinent and Latin America is responsible for the greater burden of malaria in pregnancy (1). However, by severity of morbidity and mortality, a greater burden is witnessed in areas endemic to the most virulent species of the causative agent, *P. falciparum*.

Maternal anaemia as a result of malaria in pregnancy is a well-documented adverse outcome (2). Unlike infant mortality, there is relatively little evidence for the effect of malaria related maternal mortality other than indirectly through anaemia related morbidity. Low birth weight as a consequence of malaria induced intrauterine growth restriction (IUGR) or preterm delivery is a well characterised consequence of malaria in pregnancy through direct action at the placental level or independently through malaria-associated maternal anaemia (3-6). In addition, infant mortality has been demonstrated to be aggravated by malaria exposure during pregnancy (6, 7). Increasing evidence now suggests that pregnancy associated malaria may impact on subsequent neurocognitive (8, 9) and growth (10) trajectories in early childhood independent of low birth weight.

The World Health Organization (WHO) currently advocates a universal two pronged approach for the control of malaria in pregnancy in endemic regions. This framework constitutes prompt and effective case management and use of long lasting insecticide treated bed nets (LLITNs). Intermittent preventive treatment with Sulphadoxine-Pyrimethamine (IPTp-SP) is an additional

third prong recommended for areas with moderate to high malaria transmission in sub-Saharan Africa (11).

1.1.2 Study rationale

The growing trend in resistance to Sulphadoxine-Pyrimethamine culminated in the discontinued use of SP for the management of symptomatic malaria, having been replaced by artemisinin combination therapies in recent recommendations (12). However, SP continues to remain the only drug recommended for intermittent preventive treatment for the control of malaria in pregnancy (13). The WHO recently revised its policy guidelines for IPTp-SP to be instituted to all pregnant women as early as possible in the second trimester at each scheduled antenatal visit as part of focused antenatal care until delivery, with at least one month between doses (11).

Several studies have demonstrated that IPTp-SP continues to remain effective despite growing resistance (14). Evidence from a recent study by Kayentao *et al* was integral to influencing the new recommendations by the WHO. This systematic review and meta-analysis of 7 trials with a total of 6,281 pregnancies demonstrated that increased dosing of SP was superior to the previous 2 dose recommendation (15). However, this has been in overall consideration in areas of no more than moderate malaria transmission and SP resistance. Findings in areas of higher resistance suggest that with growing resistance the current observed benefits of SP as IPTp-SP is waning (16-18). These findings represent extremes of a spectrum of the impact of SP resistance on the effectiveness of IPTp-SP. More recent unpublished findings by ter Kuile et al submitted to the WHO Evidence Review Group on malaria, demonstrated that the effectiveness of IPTp-SP decreased as the prevalence of resistant parasite genotypes increased (19).

Alternative drugs and control strategies are urgently needed for the continued efforts to successfully control pregnancy associated malaria. Several trials have been and are currently underway to evaluate various mono- and combination therapies to replace SP for IPTp. Many that

have been completed have demonstrated little or no success due to poor performance in efficacy and/ or tolerability. Increasing interest has grown for alternative strategies that involve intermittent screening and treatment (ISTp) has received particular interest. The concept of ISTp involves screening pregnant women from the second trimester, at regular scheduled antenatal visits at least a month apart, with a malaria rapid diagnostic test (RDT). Women who have a positive RDT result are then treated with an efficacious antimalarial.

We conducted a trial comparing ISTp with dihydroartemisinin-piperaquine (ISTp-DP) against IPTp-SP. The study was designed to provide the required evidence base to support policy makers with the development of potential alternative strategies that could replace IPTp-SP in areas with high SP resistance in Malawi as well as contribute to the greater body of evidence toward global recommendations for alternative control measures for malaria in pregnancy. Furthermore, because of scant and often conflicting evidence of the effect of asymptomatic malaria infections, more so those missed by RDTs, on pregnancy outcomes, and the unclear efficiency of RDTs in diagnosing infections sequestered in the placenta, the study would be able to additionally evaluate the potential impact of low density sub-patent (PCR positive, RDT negative) infections on maternal and newborn outcomes.

1.1.3 Objectives

1.1.3.1 Primary objective:

To compare the efficacy and safety of scheduled intermittent screening with malaria rapid diagnostic tests (RDTs) and treatment of RDT-positive women with Dihydroartemisinin-piperaquine (ISTp-DP) against intermittent preventive treatment with Sulphadoxine-Pyrimethamine (IPTp-SP) in the second and third trimesters on adverse birth outcomes and malaria infection at term among HIV-negative women protected by insecticide–treated bed nets. Specifically,

1. To determine if ISTp-DP compared with IPTp-SP in the second and third trimesters of pregnancy is associated with at least a 25% reduction in adverse birth outcome as composite endpoint of low birth weight, preterm birth and small for gestational age in paucigravidae.
2. To determine if ISTp-DP compared with IPTp-SP in the second and third trimesters of pregnancy is associated with at least a 50% reduction in placental malaria in multigravidae.

1.1.3.2 Secondary objectives:

The principle secondary objective from this trial that is covered in this thesis is as follows:

1. To evaluate the tolerability and safety of ISTp-DP in the second and third trimesters of pregnancy.

In addition, the following secondary objectives will also be evaluated for purposes of this manuscript, though not specified in the original trial protocol:

2. Investigate the effect of sub-patent parasitaemia by RDT on pregnancy outcomes
3. To evaluate the reliability of malaria rapid diagnostic test for the detection of active placental malaria independent of detectable peripheral parasitaemia.

1.2 Outline of this thesis

This thesis covers 3 main research discussions topics and has been arranged in 3 broad sections.

1.2.1.1 Section 1: Introduction and methods

Chapter 1 provides the a brief context in which this work has been based

Chapter 2 addresses the literature review of the research topic in greater detail and

Chapter 3 gives a description of the methods of the study from which this thesis is based.

1.2.1.2 Section 2: Results chapters

Chapter 4 describes the findings of the clinical trial that evaluated the efficacy and safety of intermittent screening and treatment with Dihydroartemisinin-piperaquine compared to intermittent preventive treatment with Sulphadoxine-Pyrimethamine for the control of malaria in pregnancy.

Chapter 5 evaluates the role of sub-diagnostic parasitaemia of common diagnostic tests on maternal anaemia and fetal outcomes.

Chapter 6 seeks to investigate the reliability of a pLDH/HRP2 combination malaria rapid diagnostic test for the detection of active placental malaria.

1.2.1.3 Section 3: Discussion, further research, overall conclusion and recommendations

Chapter 7 brings into context the brief individual discussion in the respective chapters in order to provide a perspective on the outcome of the performance of ISTp-DP against IPTp-SP and set the stage for future research. The chapter ends with an overall conclusion and policy recommendations from this work. References and annexes follow thereafter.

2 CHAPTER TWO: BACKGROUND LITERATURE

2.1 General Introduction: Malaria in pregnancy

2.1.1 Global epidemiology

Owing to the significant human capital and economic costs inflicted by malaria (20), control continues to remain a pertinent agenda of the global health community, philanthropists and multilateral development institutions alike, such as the World Health Organization (WHO), The Bill and Melinda Gates Foundation (BMGF) and the World Bank (WB) respectively. Global concerted efforts are embodied in the declaration of Millennium Development Goal 6C: To have halted by 2015 and begun to reverse the incidence of malaria and other major diseases (20). The impact of malaria percolates into the very fabric of the economic development of nations where malaria remains endemic (21), contributing to the continuous cycle of poverty. Such is the economic cost of malaria that Africa losses an estimated US$ 12 billion annually, accounting for almost 50% loss in per capita gross domestic product (22, 23). Substantial economic gains have been evident where malaria has been successfully eradicated following the efforts of the 1960's.

According to 2014 estimates (24), 3.3 billion people in 97 countries remained at risk of malaria with 36% at high risk (at least one malaria case per 1,000 persons). Approximately 125.2 million pregnancies globally are at risk of the adverse effects of malaria in pregnancy (1). The burden of pregnancy associated malaria is concentrated in the geographical areas of Latin America, the Indian subcontinent and south-east Asia, accounting for 70.5 million pregnancies in low transmission or of predominantly *Plasmodium vivax*. Sub-Saharan Africa, with 54.7 million pregnancies, bears the brunt of intense malaria transmission, mainly by *P.falciparum*, the most virulent strain causing malaria disease. Though the former three areas account for an estimated 56% of the populations at risk, sub-Saharan Africa accounts for the vast majority of the morbidity and mortality burden of malaria per pregnancy. Malaria is estimated to account for between 19% and 43% of infant Low Birth Weights (LBW), with 6% of LBW related infant deaths occurring as a consequence of malaria induced

LBW (2, 25) as well as a higher perinatal mortality rate (26). Twenty-five percent of severe anaemia in pregnancies in sub-Saharan Africa are attributed to malaria infection, with an estimated 38% complicated by any degree of anaemia instigated by malaria infection. The effect of malaria in pregnancy accounts for about 10,000 maternal deaths, and 75,000 to 200,000 infant deaths annually (27).

Pregnancy increases a woman's susceptibility to malaria infection (28), with those living in rural areas, in their first or second pregnancy, infected with HIV, and adolescents at particularly high risk. In endemic countries with stable malaria transmission, such as Malawi and much of sub-Saharan Africa, most malaria infections in pregnant women remain asymptomatic or have only mild symptoms, and are therefore undetected and untreated. Symptomatic or asymptomatic falciparum malaria infection affects the placenta and is associated with unfavourable outcomes for both mother and baby, including maternal anaemia, low birth weight (due to intra-uterine growth retardation or pre-term birth) and an increased risk of neonatal death (2). There is also increasing evidence that babies born to mothers with placental malaria infection are at increased risk of malaria infection in their first and second years of life, (29-32), and anaemia (33-35). Placental malaria has also been associated with reduced mother to child transfer of protective antibodies to infectious diseases such as measles (36). The control of malaria in pregnancy is therefore important for the health of mothers and babies and an important element of antenatal care in endemic areas.

2.2 Malawi country profile

Covering an area of approximately 118,000 square kilometers, Malawi is a small land locked country in south-east Africa. It is divided into 3 regions; North, Center and South, with 28 administrative districts. Recent estimates by the United Nations Children's Fund (37) peg the population at just under 16 million with more than half the population under the age of 18 years of age. Total fertility rate is estimated at 5.5 with 640,000 births per annum. Maternal and under five

mortality rates are relatively high, at 680/100,000 live births and 71/1,000 live births respectively though these are substantial improvements from previous years under survey. The national HIV prevalence is estimated at 10.8% with 13.6% of women in the reproductive age group being infected (38). Approximately 55% of households own an insecticide treated bed net and 94.7% of pregnant women attend at least one antenatal care visit.

2.2.1 Transmission settings

Lying between latitudes 9° and 18°S, and longitudes 32° and 36°E, the climate is generally of a tropical disposition with maritime influences, giving a strongly seasonal characteristic with the rainy season spanning between November and April, a colder dry season between May and August and a hot dry season between September and October. Between the drier and wetter months, humidity ranges from 50% to 87%. Positioned within the Great Rift Valley system, the topography of the country is characterised by undulating surface features of varying elevation, from predominant plateaus in the north of the country to plains, dotted highlands and valleys toward the south thus conferring variable weather patterns between regions which bear a strong influence on malaria transmission and infection risk. The highest risk is conferred in the low lying areas along the lakeshore, Shire Valley and central plains, that tend to be hotter, wetter and more humid, with lower risk in the highland areas (39). Malaria transmission occurs throughout the year with marked seasonal increases in malaria related morbidity and mortality.

2.2.2 Vector ecology and pyrethroid resistance

Four main species of anopheline vectors serve as principle transmission agents namely; *An. arabiensis*, *An. funestus*, *An. gambiae* s.s. and *An. Quadriannulatus* (40, 41). With human blood indices of 99.2% and 96.3% respectively, *An. funestus*, *An. gambiae* s.s are highly anthropophilic. In tandem

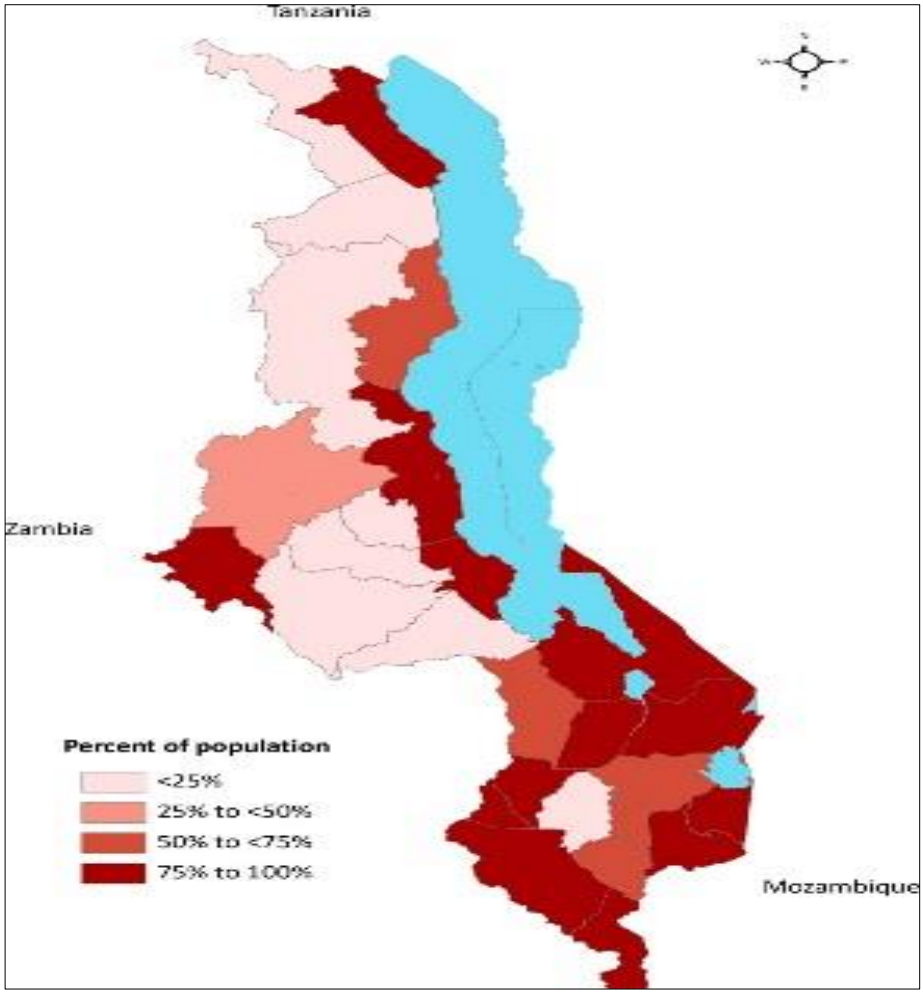
with the well-recognised perennial transmission pattern, the presence of these vectors surge during the rainy season.

Pyrethroid resistance in Malawi is classified as moderate to high. Though few, previous insecticide resistance studies have demonstrated increasing levels of resistance by main vector actors to pyrethroids and, increasing worrying, to Dichlorodiphenyltrichloroethane (DDT), (42). Despite the trend in increasing insecticide selection pressure, recent evidence demonstrates a lack of a commensurate increase in malaria transmission with continued beneficial effect of pyrethroid LLITNs, though additional implementation of insecticide based control measures appears not confer any additional benefit within the current levels of vector resistance (43, 44).

2.2.3 Parasite ecology and sulphadoxine–pyrimethamine resistance

The predominant species of Plasmodium is *P.falciparum*, occurring usually as monoinfections with the intermittent occurrence of mixed infections with other species, mainly *P.malariae* (45). Non-falciparum monoinfections are extremely rare. Resistance to Sulphadoxine-Pyrimethamine is wide spread with near saturation of quintuple mutants (46).

Figure 2.1 Map of proportion of the population under highest transmission intensity by district (predicted PfPR2-10, 40-50%. 2010)



(Bennett et al., 2013)

2.3 Malaria and HIV coinfection in pregnancy

Malaria and HIV infection commonly overlap in their geographic distribution (47) and whose intersection is more pronounced in pregnancy with significant consequences owing to the synergy in effects between the two diseases. An estimated 1 million pregnancies are complicated by coinfection with Malaria and HIV infection in sub-Saharan Africa every year (48).

By compromising the immune system though the reduction in CD4 + cells and thus the cell mediated immunity, HIV infection substantially increases susceptibility to infection and severity of disease in pregnancy with greater adverse pregnancy and birth outcomes such as maternal anaemia and low birth weight (48). HIV infection consequently annuls the gravidity dependant immunity against malaria, rendering women of higher gestational orders vulnerable to the severity and risk of malaria infection as paucigravidae (49, 50). A review of the burden of HIV-1 and malaria co-infection found that HIV infected pregnant women consistently experienced higher placental and peripheral parasitaemia as well as malaria related morbidity and adverse birth outcomes (51).

Malaria in the face of co-existing HIV infection has been demonstrated to confer greater viral replication (52, 53) and resultant higher maternal viral loads (54).This confers the possibility of increasing the risk of mother to child transmission of HIV prenatally (52), though the body of evidence around this effect remains polarized with some studies not showing any additional risk conferred for mother to child transmission of HIV-1 (52, 55). In-vivo studies have however suggested that a possible mechanism for the facilitation of HIV transmission perinatally may be mediated through increased HIV-1 replication in placental cells instigated by TNF – alpha following a cell mediated response to VAR2CSA – CSA binding of infected erythrocytes in the intervillous vasculature of the placenta (56).

2.4 **Maternal undernutrition and malaria in pregnancy**

Nutritional status is a key determinant in pregnancy outcomes (57-59). Acute malnutrition demonstrates marked seasonality with higher incidence during the rainy season, which coincides with cultivation and food insecurity, as compared to the post rainy and dry season that coincide with harvests (60). The impact of this seasonal variability confers a similar seasonal trend on low birth weight. Peaks in LBW due to small for gestational age are evident at the end of the rainy season, culminating from low maternal energy intake during the cultivating season. However, this

observation may also arise as a result of chronic sub-patent malaria infections over the same period which also is characterised by high malaria transmission.

Studies of the relationship between maternal nutritional status and malaria susceptibility in pregnancy are few. In children under the age of five years, micronutrient deficiencies and gross undernutrition increase the burden of malaria related morbidity and mortality (61). Maternal undernutrition and malaria have been noted to interact in increasing the risk of IUGR (62) suggesting adverse birth outcomes could be improved through a coupled approach to nutrition and malaria intervention during pregnancy.

2.5 Malaria in pregnancy as a direct cause of maternal mortality

Traditionally, malaria in pregnancy has not been considered as a major direct cause of maternal mortality. However, evidence from a previous study by Menendez and colleagues in Mozambique has challenged this prevailing doctrine (63) by concisely evaluating the role of malaria which has lacked in previous maternal mortality autopsy studies. In this prospective autopsy study on causes of maternal deaths, evaluation for malaria parasitaemia, including CNS examinations and histology of multiple organs, were conducted and found evidence of malaria infection in 66.2% of the autopsies. 10.1% of the women (n = 14) were determined to have died due to severe malaria, more than a third of whom were due to cerebral malaria.

A more recent nationwide autopsy study conducted in Mozambique by Singh et al demonstrated a higher direct contribution of malaria to maternal mortality (64). Malaria accounted for 23.1% of maternal deaths in a setting where only 42.3% of pregnant women slept under an insecticide treated net and 19.6% received at least two doses of Sulphadoxine-Pyrimethamine for intermittent preventive treatment.

These two studies provide the most concise findings of the direct contribution of malaria to maternal mortality which has lacked in previous publications. Their findings highlight the

preventable contribution of malaria to maternal mortality which has been otherwise grossly underestimated.

2.6 Aetiology and pathophysiology of malaria in pregnancy

Of the known 120 species of Plasmodium, to date there are only 5 species known to cause malaria in humans: *P.malariae*, *P.falciparum*, *P.ovale*, *P.vivax* and more recently, a previously predominantly simian species, *P.knowlesi*, reported from Asia. With the exception of *P.knowlesi*, each species has a characterised virulence in the general population and particularly in pregnancy, with falciparum being the most aggressive, followed by vivax. The method of transmission of P.*knowlesi* from simian hosts has remained elusive but the impact by this strain is set to increase due to transmission within the human pool (65, 66). It has been postulated that P.*knowlesi* is likely to confer similar adverse pregnancy outcomes as *P.falciparum* and P.*viax* but the magnitude of risk and severity are subject to further investigation (67).

2.6.1 Host malaria immunological responses in pregnancy

Immune responses to *P.falciparum*, which harbors the greatest virulence and confers the greatest burden of malaria morbidity and mortality globally, are the best characterised of all Plasmodium species causing malaria. The natural acquisition of protective immunity to *P.falciparum* is largely dependent on transmission intensity and develops as a result of the slow attainment of a broad spectrum of immune responses to several antigens, including members of the Plasmodium falciparum erythrocyte membrane protein 1 (P*f*EMP1) family. In pregnancy, this acquired immunity to blood stage malaria is reduced and further susceptibility to severe *P.falciparum* malaria infection is conferred, especially in the placenta due to a lack of immunity to placenta specific cytoadherence proteins. Immunity to placental cytoadherence only develops over the course of subsequent pregnancies which results in the reduced occurrence of malaria associated adverse outcomes in the mother and foetus over several pregnancies (68-70).

2.6.1.1 Cellular mediated responses

Due to the need to taper immune responses in pregnancy so as not to reject the fetal allograft, Th2 subset of T helper cells, supported by the hormones progesterone and oestrogen (71), modulate a predominantly systemic anti-inflammatory response. This is mainly achieved through transforming growth factor β (TGF-β) and interleukins - 4, 5, 10 and 13. An initial pro-inflammatory sequence by Th1 cells via interleukin (IL)-2, tumour necrosis factor α (TNF-α), and interferon gamma (IFN-γ) is essential being essential in the early phases of pregnancy to facilitate implantation (72) but is later dampened by the predominance of the anti-inflammatory response.

A high prevalence of malaria parasitaemia between weeks 10 and 12 of pregnancy (73) coincides with peak Th2 cytokine levels suggesting that the anti-inflammatory milieu during this period confers a state of temporary reduced immunity. Infection with malaria stimulates a Th1 mediated immune response, which is elicited in the acute phase of the infection to clear the parasites (74). These responses when localised in the placenta as a result of sequestration of infected erythrocytes (75) via the binding of the VAR2CSA antigen expressed on the surface of IEs, and chondroitin sulphate antigens expressed on the walls of the intervillous vasculature, stimulates localized expression of TNF-α (76) by macrophages. This initial reaction, coupled with expression of Migration Inhibitory Factor (MIF) from the syncytiotrophoblast, further stimulates macrophage recruitment leading to a vicious cycle of inflammation, consequently compromising the structural integrity of the placenta.

Unlike TNF-α, IFN-γ produced in response to malaria infection is not associated with adverse pregnancy outcomes (77). IFN-γ is postulated to confer a gravidity dependant protection reliant on the maternal ability to limit levels within non-pathological concentrations (78). Multigravidae undergo a rapid peak of IFN-γ levels in response to malaria infection that subsequently drops as opposed to sustained peaks seen in primigravidae (79).

2.6.1.2 Humoral mediated responses

Humoral response mechanisms are orchestrated by cell mediated immune responses to malaria infection via the activation of B cells by Plasmodium specific CD4 + T cells during blood stage infection (80), leading to the production of antibodies that prevent erythrocyte infection, block cytoadherence and promote phagocytosis by macrophages (74, 81). The placental cytoadherence phenomenon exhibited by *P.falciparum* is mediated by ligands expressed on the surface of infected red blood cells and surface adhesion molecules expressed on the placental vascular endothelium such as intercellular adhesion molecule 1 (ICAM-1), CD36, chondroitin sulphate A (CSA) and hyaluronic acid (82, 83).

Immunity against malaria in pregnancy, in particular, against placental sequestration mediated by VAR2CSA, is largely mediated by the evolution of humoral immunity over subsequent pregnancies with exposure to malaria (84). Immunity acquired prior to pregnancy is not protective against placental sequestration (85). This pregnancy-specific humoral mediated protection is conferred by immunoglobulin G (IgG) subclass of antibodies specific to the VAR2CSA ligand (86). However, antibodies to merozoite antigens, which are not specifically associated with pregnancy, also follow a parity dependent trend in conferring malaria protection.

2.6.2 Effect of malaria infection on pregnancy outcomes

2.6.2.1 Early peripheral parasitaemia and its impact on placental development

With peak malaria prevalence in pregnancy occurring prior to the full establishment of the placenta between 10-12 weeks of gestation (73), the induction of pro-inflammatory cascade may adversely impact the subsequent growth and expansion of placental vasculature between 16-18 weeks through abrogated trophoblast invasion and migration (87). Recent in-vitro models have demonstrated reduced trophoblast invasion and migration when exposed to plasma from women infected with *P.falciparum* than plasma from uninfected women (87). These findings were further

suggested by the lower levels of trophoblast promoting hormones and higher concentration of inhibitory factors in *P.falciparum* infected plasma. In the same study, the well characterised virulence of *P.falciparum* infection was further evidenced as retarded trophoblast migration was not observed with plasma from women infected with P.*vivax*.

In mid-gestation, malaria infection results in the dysregulation of vascular development and angiogenesis regulated by Angiopoietins 1 and 2 (ANG-1 and ANG-2) in murine models (88). Furthermore evidence from a case controls study in 492 women showed that malaria in pregnancy was associated with derangement of the complement C5a system which, when blocked in mouse models, was associated with improved placental development and function (89).

2.6.2.2 Placental sequestration and infection

The hallmark of Plasmodium falciparum infection in pregnancy after the placenta is established is the sequestration of infected erythrocytes in the placenta. This is mediated by the binding of VAR2CSA proteins expressed on the surface of infected erythrocytes to chondroitin sulphate-A receptors expressed in the placental vasculature (90). Pro-inflammatory mediators elicited in response to the expressed antigens lead to infiltration by inflammatory cells with consequent pathological changes to the placental architecture characteristic of placental malaria on histological examination (91-93). Recently, P.*vivax* has also been shown to bind to placenta ligands in-vitro (94) though thus has not been demonstrated in placental autopsies. Placental lesions are less pronounced in women infected with P.*vivax*, lacking the presence of the characteristic placental sequestration or hemozoin pigment of *P.falciparum* infection (95).

Localized inflammatory reactions in response to the sequestration of *P.falciparum* infected erythrocytes result in adverse birth outcomes (96) thought to be mediated through impaired nutrient invasion, dysregulation of growth regulating hormones and retarded placental vascular development (6, 97, 98). Furthermore, the propensity of this act of sequestration makes the diagnosis

of malaria infection based on peripheral blood examination difficult and thus leads to missed malaria infections (99).

As with systemic malaria antigen exposure, immunity to placental sequestration is largely unsterelising but develops with repeated exposures, conferring some degree of protection. As such, the consequences of placental infection are greater in paucigravidae than multigravidae (100) with this relationship being more pronounced in high transmission settings (101).

2.6.2.3 Malaria induced anaemia in pregnancy

An estimated 400,000 pregnancies in sub-Saharan Africa are complicated by moderate to severe malaria-anaemia (102). Physiological adaptations during pregnancy predispose toward unfavorable fluid and iron balance, and consequentially to a higher risk of anaemia. Fluid retention mediated by argenine vasopressin (AVP) (103) results in an increased intravascular volume which leads to hemodilution and physiological anaemia. The increased metabolism of iron and folate mediated by fetal hepcidin (104, 105) further increases the demand for iron to sustain the maternal intrinsic requirements for erythropoiesis and fetal metabolism, which in instances of intake below the recommended daily allowance in pregnancy leads to iron deficiency anaemia (106) with consequent adverse pregnancy and birth outcomes.

Malaria induced anaemia is characterised by two main disease processes; parasite mechanisms infringing on erythropoiesis, iron metabolism, and infected erythrocyte sequestration in the spleen and placenta. The inflammatory cytokines induced by parasite infection has been well recognised in inhibiting erythropoiesis, with further suppression caused by the accumulation of heamozoin, a parasite by-product of haem metabolism, in the bone marrow (107). Red cell destruction as the parasite migrates from the schizont to merozoite stages of the life cycle appears to play a less significant role in the pathogenesis of malaria anaemia (108).

Organ sequestration plays a significant role in the genesis of anaemia syndromes in malaria in pregnancy. The red pulp zone of the spleen demonstrates intense phagocytic and free radical by macrophage activity in trapping and destroying parasitised erythrocytes much in a similar manner as senescent and aberrant red blood cells (109). This action may be mediated as a result of membrane and cellular transformations in the red blood cell following parasitisation, which may include loss of self-surface markers such as CD47, or the expression of foreign antigens on the red cell surface that promotes recognition, cytoadherence and phagocytosis in the spleen, with this high functionality of the spleen in acute infection characterised by spenomegaly (110).

2.6.2.4 Adverse fetal outcomes

Low birth weight (birth weight less than 2.5kg) is one of the most well recognised consequences of malaria in pregnancy on the foetus, with a significant bearing on latter survival and development in childhood. This arises as a consequence of two different mechanism that may occur simultaneously or independently as a result of malaria infection in pregnancy. Due to the associated immunological mechanisms of each pathway, the relative contribution to LBW evidently follows the distribution of malaria transmission. In areas of unstable transmission where pregnant women have not developed a robust immunity to malaria, systemic malaria infection is characterised by fever, precipitating preterm birth and consequently, low birth weight (111, 112). In contrast, the burden of LBW in areas of stable malaria transmission is largely due to intrauterine growth restriction due to placental insufficiency and maternal anaemia induced by chronic and largely asymptomatic malaria infection (113). As such, there is a higher proportion of LBW characterised by small for gestational age (SGA).

2.6.2.5 Post-birth ramifications of malaria in pregnancy independent of low birth weight

There is mounting, albeit inconclusive, evidence that maternal malaria infection increases infants' susceptibility to malaria infection through the pre-natal exposure to malaria antigens and

possible immune-sensitisation. An early study conducted in Cameroon found no significant difference in the frequency of malaria between infants born to mothers with placental malaria and infants born to mothers without placental malaria during the first twenty four months of life (114). Findings from an analysis of a sub-set of the cohort suggested that increased infant susceptibility to malaria may be due to immunologic tolerance or congenital transmission of parasites (115), the later hypothesis which was supported by findings from a later study in Kenya where children exposed to malaria in-utero acquire a tolerant phenotype to blood-stage antigens that persists into childhood and is associated with an increased susceptibility of the child to malaria infection and anaemia (116). Importantly, these effects are independent of low birth weight; i.e. they also occur in term children with normal birth weight.

A subsequent study conducted in Muheza District in Tanzania found that infants born to mothers who had evidence of placental malaria at delivery were 41% more likely to experience their first parasitaemia at a younger age compared to those who were born from mothers who did not have placental malaria at delivery (117). However, the increased risk was confined to infants born to multigravid women and not primi and secundigravid women which was surprising considering the known high risk of malaria in primigravidae and high incidence of post-neonatal infant mortality of infants born to malaria infected primigravid mothers with malaria (118-120). A more recent study from Gabon provided further evidence suggesting that malaria in pregnancy increases early childhood susceptibility to malaria infection. It found that offspring of mothers with placental *P. falciparum* malaria infection were about twice more likely to have clinical malaria in the first 30 months of life than those born to uninfected women. In addition, the median parasite density was more than three times higher in infants born to malaria-infected mothers (121, 122).

Despite these suggestive findings of a biological mechanism conferring an increased risk of malaria morbidity in early childhood stemming from in-utero exposure, this association has been alternatively attributed to being a function of economic status and transmission intensity. Findings

from an infant cohort study conducted in a high transmission area in Ghana, born to mothers with and without evidence of placental malaria at delivery, showed no difference in the incidence of malaria in the first 12 months of life irrespective of gravidity, with the exception of infants from primigravid mothers who did not have placental malaria (a proxy for localised low transmission), who had a lower incidence of malaria infection and morbidity than the 3 other groups. There was however a marked association of economic status with the risk of malaria infection, with infants from poor households having a doubled higher risk than their counterparts from better socioeconomic classes (123). However, this comparison does not take into account the older age of children in the prior studies and thus may be a reflection of risk factors in the first 12 months of life, with the immunomodulatory mechanisms stemming from in-utero exposure playing a significant role in the later years of childhood. More so, additional recent findings from a study done in Cameroon points back to biological mechanisms that may stem from intrauterine exposure with IPTp-SP use and month of birth having increasing the risk of later susceptibility effect (124).

Malaria in pregnancy has been demonstrated to be a significant factor in early childhood survival in malaria endemic areas. Due to the direct effects on conferring higher risk of low birth weight, malaria in pregnancy indirectly increases the risk of infant mortality mediated by low birth weight (7, 125) . Owing to increased susceptibility to malaria infection as a result of in-utero exposure to malaria antigens, children exposed to malaria in pregnancy also succumb to a greater risk of malaria anaemia (9). Post birth anthropometric development has also been elucidated to be influenced by malaria exposure in-utero through the dependent effect of increased malaria susceptibility in childhood (126). Invariably, a vicious cycle exists, with malnutrition playing a further role in conferring higher risk of malaria infection and overall mortality in infants born to mother with malaria during pregnancy (127, 128).

Stimulation of the pro-inflammatory cytokine cascade due to exposure to malaria antigens directly (in congenital malaria though rare) or indirectly (in placental and maternal peripheral

malaria) as well as placental inflammatory cytokines produced in pregnancy associated malaria, may have a potentiating effect to adverse neurocognitive outcomes in infants in pregnancy associated malaria other than low birth weight (8). To this extent, it is estimated that infants born to primigravid and secundigravida mothers are at greater risk of neurocognitive impairment through this pathway as such mothers are more susceptible to malaria infection. Indirectly related to the occurrence of malaria n pregnancy, antimalarial therapies in chemoprophylaxis and treatment programmes in pregnancy have been widely studied to determine the safety and efficacy of antimalarials in pregnancy, reduction of maternal malaria associated morbidity and mortality and adverse birth outcomes. However, there is little evidence that has evaluated the impact of prolonged in-utero exposure to antimalarials on neurocognitive development.

2.6.3 Malaria effect outcomes for clinical research endpoints

In the absence of HIV co-infection, clinical outcomes to measure the effect of interventions against malaria in pregnancy must take into consideration the sensitivity of the outcome by the influence of gravidity through acquired immunity (129, 130). Paucigravidae are more susceptible to aggressive pathophysiological consequences of malaria infection leading to greater risk of adverse fetal outcomes including, low birthweight, preterm birth and intrauterine growth restricted mediated small for gestational age (2), making them ideal indicators for effect. Malaria infection in multigravidae remains largely low density and asymptomatic with a lower significance on adverse fetal outcomes. However, persistent infections, both in the peripheral blood and sequestered in the placenta, have a strong significance on ex-utero consequences of new born morbidity and mortality (31, 131). As such, peripheral and placental malaria infection is an ideal indicator for effect amongst multigravidae.

Owing to the significance of these gravidity dependent outcomes, the magnitude of effect change to influence policy would differ. Conservative changes, such as a 25% effect size, in immediate

effect indicators in paucigravidae may be sufficient to influence policy owing to the weight of these outcomes. In comparison, the later effects of malaria infection, more pronounced in multigravidae, may require larger arbitrary effect sizes, such as a 50% effect change, to influence policy. Taking into consideration that individual outcomes are clinically meaningful, are of similar importance to pregnancy outcomes and do not exert any negative influence between each other , pooling them into respective gravidity specific primary composite endpoints would provide for greater statistical efficiency with respect to the anticipated effect size.

2.7 Implications of different malaria transmission intensities on the distribution and burden of malaria in pregnancy

Owing to the diverse transmission spectrum of malaria transmission, implications for consequences of malaria infection in pregnancy are equally diverse mainly dictated by the acquisition of antimalarial immunity through episodes of exposure (132). Pregnant women in low and unstable transmission regions have less developed antimalarial immunity and thus morbidity is mainly characterised by acute severe malaria syndromes such as severe anaemia and cerebral malaria, with the consequent high occurrence of adverse pregnancy outcomes and fetal and maternal mortality due to malaria (133).

Though sterilizing immunity is not obtained, repeated malaria exposure often confers a more robust antimalarial immune system in pregnant women in areas of more stable transmission (134) except in instances of immune suppression syndromes such as HIV infection (51, 135). In such scenarios, malaria infection is less patent and exists as a more chronic infection with low grade parasitaemia or often sub-patent and recurring. Though clinical syndromes are less acute, such infection status is sufficient to cause significant morbidity in pregnancy and adverse birth outcomes (25, 136, 137)

2.8 Pregnancy: A possible reservoir for malaria transmission

Mature gametocytes are the sole transmission entity of malaria from human hosts to mosquito vectors. Commensurate with their greater susceptibility to malaria infection, there has been growing interest in the facilitation of malaria transmission by pregnant women especially in the growing context of Sulphadoxine – Pyrimethamine resistance. A recent study conducted in Blantyre, Malawi demonstrated significant gametocyte carriage by pregnant women at their first antenatal visit with IPTp-SP having no effect on the subsequent risk of gametocytemia in the later course of pregnancy (138).

The use of Sulphadoxine – Pyrimethamine has been demonstrated to be associated with increasing gametocyte carriage in both pregnant and non-pregnant populations. From 1998 to 2002, a study conducted in Mpumalanga Province, South Africa demonstrated a more than 7-fold increase in the duration of gametocyte carriage associated with the increasing prevalence of *dhfr* and *dhps* Sulphadoxine-Pyrimethamine resistance mutations (139). A more recent study in a cohort of pregnant Nigerian women showed that IPTp-SP may actually predispose toward gametogenesis and gametocyte carriage (140) and recommended increased use of (LL)ITNs for the reduction of malaria transmission facilitated by pregnant women in malaria endemic regions where IPTp-SP is one of the strategies of choice for the control on malaria in pregnancy.

Current recommended use of artemisinin combination therapies for malaria case management ameliorates the risk of pregnant women in facilitating gametocyte transmission. Based on guidelines for the management of uncomplicated malaria, published in 2010 by the WHO, artemisinin combination therapies are pivotal for the management of uncomplicated malaria (141) from the second trimester of pregnancy. Beside their efficacy in clearing asexual parasitaemia, ACTs also have gametocidal properties, reducing carriage 4-fold (142). However, largely persistent asymptomatic infections in pregnancy mean that the majority of gametocyte carriage will not be

treated with ACTs and thus continued exposure of asymptomatic parasitaemia to SP during IPTp largely means pregnant women may become significant reservoirs for malaria transmission in the current era of growing SP resistance.

2.9 Control of pregnancy associated malaria: Current Recommendations, Strategies and Challenges

The World Health Organization (WHO) currently recommends a three pronged approach for the control of malaria in pregnancy; case-management of symptomatic malaria; providing long lasting insecticide-treated bed nets (LLITNs) to reduce exposure to infective mosquito bites; and intermittent preventive treatment of malaria in pregnancy (IPTp).

2.9.1 Insecticide Treated Bed nets (ITNs)

Early individual and cluster randomised trials have established the benefits and cost effectiveness of insecticide treated bed nets in reducing the impact of malaria infection in pregnant and non-pregnant populations, and reducing malaria transmission, with several meta-analyses consolidating these conclusions. Early trials conducted in Africa from the late 1980's to the early 1990's unequivocally demonstrated the protective efficacy of ITNs against malaria morbidity and mortality in children of up to 62% (143-145), findings which were further strengthened in more recent studies (146). Consolidation of these findings in a meta-analysis further supported the evidence of ITNs as a cost-effective strategy for the prevention of malaria in children (147, 148). ITNs not only reduced malaria associated morbidity and mortality in children but also reduced malaria transmission at the population level through reduced vector populations (149-152). The protective effect of ITNs however may be transmission dependent as demonstrated by a recent study conducted in Myanmar, in an area with unstable and highly seasonal transmission, where no additional benefit was conferred by the use of ITNs (153). Additional concerns have been raised of the risk of rebound mortality in older child hood age groups with extended ITN use and the concomitant overall cost

effectiveness of ITNs (154) but these concerns have largely remained unsubstantiated in further studies (151, 155, 156).

Following the encouraging efficacy findings of ITNs on malaria morbidity and mortality in children, several subsequent trials were conducted, evaluating the impact of ITNs on malaria associated pregnancy outcomes. An early study amongst primigravidae eastern Kenya, characterised by lower malaria transmission with an estimated entomological inoculation rate of 10 infective bites per person per year, demonstrated no beneficial impact on several outcomes including birthweight and severe anaemia (157). However, a later study in western Kenya with more intense transmission showed ITNs had significant protective efficacy on anaemia, low birthweight, placental malaria and maternal malaria at delivery, with reduced incidences of malaria infection (158). The contradiction in findings between the two studies strongly suggested the influence of local transmission intensity on the efficacy of ITNs on pregnancy outcomes, similarly to paediatric populations. Evidence from several meta-analyses have demonstrated that ITNs overall are beneficial for the protection of pregnant women against malaria and improvement of pregnancy outcomes (159). One of the most recent and comprehensive meta-analyses undertaken on the protective effect of antimalarial interventions in pregnancy and related pregnancy and infant outcomes collated data from 32 African countries. The study demonstrated significant reductions in malaria associated morbidity and adverse birth outcomes with the use of ITNs under operational conditions (160). These results were supportive of findings from an earlier systematic review of five ITN trials (161) which demonstrated reduced adverse live birth outcomes as well as maternal parasitaemia and anaemia with ITN use. Despite concerns of rapidly expanding pyrethroid resistant vectors, the ITNs appear to provide continued benefit against the adverse effects of malaria in children and pregnant women as evidenced from recent findings from a study in Malawi where non-ITN users were 1.5 times more likely to have malaria infection and ITNs reduced the incidence of malaria infection by 30% (162).

Long Lasting Insecticide Treated Bed nets for the prevention of malaria in pregnancy continue to be aggressively advocated by the WHO (163) and have been the subject of greater resource mobilization in malaria prevention efforts. An estimated 214 million LLITNs were distributed in 2014 (24) with a large proportion in the decline in malaria associated morbidity and mortality in pregnancy being attributed to LLITNs in light of rising SP resistance (164, 165). Indeed owing to this efficacy, the WHO strongly advocates for universal coverage of LLITNs instead of previous limitations only to vulnerable populations (under 5 children and pregnant women) (165).

2.9.2 Effective case management

Despite robust efforts to prevent malaria infection by use of ITNs, cases of clinical malaria are inevitable. It is within such incidents that prompt and effective case management with an efficacious antimalarial drug is required. Effective case management entails two principles; accurate diagnosis and treatment with an efficacious antimalarial.

Proven parasitaemia is a prerequisite for the prescription and administration of antimalarial drugs (141, 166, 167) to prevent unwarranted drug pressure that would cultivate drug resistance of current first line antimalarial agents. Examination of thick blood films was previously the recommended standard for diagnosing malaria. However, due to poor infrastructure, limited expertise and being time consuming, light microscopy has posed a significant hindrance to access to effective case management in many resource limited settings. The advent of lateral flow immunochromatographic assays for malaria has provided the much needed reprieve for poor resource settings, mostly characterised by high caseloads, allowing equitable access to effective malaria treatment. More commonly known as malaria Rapid Diagnostic Tests (mRDTs), these novel assays are quick and simple to use, having proven to be as reliable as traditional microscopy for the diagnosis of malaria (168) and suitable in settings where microscopy presents a bottleneck to access to effective management of clinical malaria.

Current treatment guidelines for uncomplicated malaria in the first trimester of pregnancy by the WHO recommend a seven day course of quinine and clindamycin or artesunate in place of quinine if the initial regimen fails, with an ACT permitted if it is the only treatment available (141). Within the second and third trimesters, a regionally appropriate ACT is recommended or alternatively, first trimester regimens may be prescribed. ACTs have been shown to be efficacious for treatment of falciparum and non-falciparum species of Plasmodium (169, 170). However, despite the wide spread use of ACTs in pregnancy, clinical data on safety and efficacy in early pregnancy is limited and inconclusive (171). Recently completed treatment trials in pregnancy in Africa are expected to provide further insight into the safety and efficacy the 4 fixed-dose combination ACTs available to date in the 2nd and 3rd trimester (172).

2.9.2.1 Dihydroartemisinin-piperaquine (DHA-PQ) in pregnancy

Treatment trials in non-pregnant adults and children have shown DHA-PQ to be highly effective and well tolerated (173). Minor adverse events associated with DHA-PQ included nausea, vomiting, loss of appetite, diarrhoea, abdominal pain, and headache, dizziness, and sleep disturbance (173). A case series study in Thailand using DHA-PQ to treat pregnant women after treatment failure with quinine suggested that DHA-PQ was effective in pregnancy at the standard adult dose (174). Significant experience with DHA-PQ in the second and third trimester from southern Papua New Guinea and Indonesia, where the drug has been used as first line treatment in the second and third trimesters of pregnancy, has shown dihydroartemisinin-piperaquine to be very effective, safe and well tolerated in the second and third trimesters of pregnancy.

Despite animal studies having demonstrated artemisinins to be embryotoxic and potentially teratogenic in very early pregnancy in all animal species tested, similar findings have not been noted in humans as there remains a paucity in documented effects of exposure in the first trimester. In over 1,000 documented pregnancies, no adverse effects on the mother or foetus were noted when

dihydroartemesinin-piperaquine was administered in the second or third trimester of pregnancy (12).

Reproductive toxicity studies in Australia (Davis, unpublished observations) and more recently by Sigma Tau (Medicine for Malaria Venture, personal communication) have not raised safety concerns with piperaquine. A lengthening in the duration of labour in rat models has been observed (Medicine for Malaria Venture, personal communication), but this has not been confirmed in humans in Thailand (Nosten, personal communications). Despite raising bioavailability and antimalarial efficacy (175), concerns of piperaquine cardiotoxicity have been raised following either co-administration with fat or repeated dosing that may result in elevated risks of prolongation of the QT interval (QTc) (176, 177).These concerns have not been validated in studies conducted in paediatric, non-pregnant and pregnant populations when administered at recommended doses (178-180). However, significant QTc prolongation with a grade 1 cardiac event has been observed in a sample of healthy male volunteers when the treatment regimen was shortened from 3 to 2 days owing to the effective rapid raise of the piperaquine plasma concentration (181).

2.9.3 Intermittent preventive treatment with sulphadoxine-pyrimethamine

Intermittent preventive treatment in pregnancy (IPTp) is currently recommended for HIV-negative women in all areas with stable moderate to high transmission of malaria (13). The strategy consists of administration of treatment doses of an efficacious antimalarial drug at predefined intervals at least a month apart during the second and third trimesters of pregnancy, regardless of the presence of malaria parasitaemia. The previous policy was to provide two doses as part of routine antenatal care but this has since been revised by the WHO to a dose at every antenatal visit from quickening up to 36weeks, at least 4 weeks apart (182) . The strategy is thought to work by providing intermittent clearance or suppression of parasites in the placenta, and preventing new infections from occurring.

Sulphadoxine-Pyrimethamine (SP) is the only drug currently used for IPTp. It has a profile that makes it highly suitable for this use. SP is very well tolerated and safe in the second and third trimester of pregnancy for mother and foetus (14). SP is also widely available, cheap, and can be given as a single dose. IPTp-SP is not recommended for HIV-positive women. WHO recommends that HIV-positive women receive co-trimoxazole prophylaxis for HIV-related infections (183). As co-trimoxazole and SP are both sulpha-containing drugs, SP is contra-indicated in HIV-positive women receiving co-trimoxazole. However, co-trimoxazole itself has some antimalarial properties and therefore serves to protect pregnant women with HIV infection from malaria infection (183).

As part of efforts to halt and reverse the incidence of malaria by 2015, and achieve a further 90% reduction by 2030 as outlined in the previous Millennium Development Goals and current Sustainable Development Goals respectively, coverage of malaria interventions, including IPTp-SP, were earmarked for at least 80% coverage (184). Despite the simplicity of the strategy, operational delivery has largely remained ineffective in many countries with an estimated 48% of pregnant women in endemic settings not receiving any dose of IPTp-SP in 2013 (24). Findings from a synthesis and meta-analysis of national survey data between 2009 and 2011 from 27 countries showed that despite relative improvements in coverage of 2 dose IPTp-SP, the achieved levels remained below international recommendations (185). Estimates from Malaria indicator surveys in Malawi in 2012 and 2014 have shown 54% and 64% of eligible pregnant women receiving at least 2 doses of IPTp-SP (186, 187). In light of the recent amendment of recommendations by the WHO for IPTp-SP to be administered at each antenatal visit from the 16 week of gestation, at least 4 weeks apart, indicating an upward recommended dosing of 3 or more doses (3, 11), there remains concern on the ability of national control programmes to scale up the coverage of the new recommendations stemming from a background of below par coverage of the prior lower dosing regimen as reflected by coverage of less than 10% in a recent systematic analysis of 58 household surveys from sub-Saharan Africa (188).

2.9.4 Other strategies for the control of malaria in pregnancy: Unsanctioned policies, a sign of desperation

The WHO has no other recommendations for the control of malaria in pregnancy other than the three pronged approach. However, this renders prevention policy in areas of unstable transmission, where P.*vivax* is predominant or areas of reducing transmission, vulnerable to lack of guidance where IPTp-SP may not be applicable. Growing resistance to SP in areas of stable transmission has also brought the sustainability of IPTp-SP into question.

To date, Indonesia is the only country that has implemented Single Screen and Treat in pregnancy (SSTp) as a policy in place of IPTp-SP. The strategy involves screening all pregnant women at their first antenatal visit and treating those with malaria. Thereafter, passive case detection is employed and women are only treated for symptomatic malaria. The efficacy of this strategy has no supportive evidence and currently for the first time in Asia, a clinical trial evaluating IPTp with Dihydroartemisinin-piperaquine and Intermittent Screening and Treatment with Dihydroartemisinin-piperaquine are being evaluated as alternative options in this region (189), the result of which are anticipated in 2016.

As a result of declining malaria transmission and subsequent reductions in the prevalence of placental malaria or concerns over SP resistance, some countries have abandoned IPTp–SP. Despite WHO recommendations, Rwanda discontinued IPTp–SP as a policy in 2008 due to increasing SP resistance and declining malaria prevalence. A recent study in Zanzibar has questioned the continued implementation of IPTp–SP in light of low prevalence of peripheral parasitaemia (190), with intensified surveillance, continued vector and transmission control with LLITNs and effective case management being recommended.

2.10 Determinants of the effectiveness of Intermittent Preventive Treatment with Sulphadoxine-Pyrimethamine

The frequency of dosing has been unequivocally demonstrated to improve IPTp–SP efficacy across transmission settings. In a randomized control trial comparing 2 vs 3 doses of IPTp–SP in an area of highly seasonal malaria transmission and low SP resistance, adding a third dose of IPTp–SP halved the risk of adverse pregnancy outcomes in all gravidities (191). A meta-analysis of 7 trials with over 6,000 pregnancies concluded 3 or more doses of IPTp–SP improved birth outcomes significantly in comparison to 2 doses (15).

In areas of high transmission with moderate SP resistance, at least two doses of IPTp-SP were associated with significant protective efficacy which was more pronounced in paucigravidae (192). In this retrospective birth outcomes study in Mansa, Zambia, at least two doses of IPTp–SP showed a reduction in LBW and any malaria infection.

A comparative study of the effect of IPTp–SP in two different transmission settings in Tanzania comprehensively underscores the transmission dependency of the efficacy of IPTp–SP. Using a prospective observational study design in two areas of differing transmission, one high and one low, IPTp–SP was effective in lowering the risk of placental malaria in the high transmission setting with no significant effect in the low transmission area (OR 0.2; CI 0.06-0.7; P = 0.015 vs OR 0.4; CI 0.04-4.5; P = 0.478 respectively). However, IPTp–SP use did not confer any protection to maternal anaemia or LBW (193).

Work in HIV sero-positive pregnant women has however remained controversial. Earlier work in Malawi comparing 2 doses against monthly dosing of IPTp–SP in HIV sero-positive and seronegative women demonstrated greater efficacy of monthly dosing than the standard 2 doses in the HIV sero-positive cohort (194). However, a study in Zambia showed no significant effect of increased dosing on any maternal or birth outcomes (75). These differences may be attributable to differing transmission intensities in the two study settings.

2.11 Sulphadoxine –Pyrimethamine resistance: mechanism and drivers of an ever growing challenge for malaria control in pregnancy

Drug resistance has been a recurring impediment to malaria control efforts, in particular for the control of malaria in pregnancy. Prior to the advent of IPTp-SP, weekly chloroquine chemoprophylaxis was the mainstay of malaria prevention in pregnancy (195). The rapid spread of chloroquine resistance over the next two decades from the 1950's, following emergence of resistance along the Thailand – Cambodia border (196) rendered chloroquine inefficient for case management or prophylaxis in pregnancy in malaria endemic areas. Following notable improvements in pregnancy and birth outcomes through several randomized trials and observational studies in the 1990's (197, 198), IPTp–SP was adopted as a replacement strategy.

However, rapidly expanding resistance, like its predecessor chloroquine, rendered SP ineffective for case management, with subsequent recommendations for withdrawal of SP for active case management and replaced with ACTs. Despite high treatment failure rates due to resistance, SP has remained a critical component for malaria control in pregnancy strategy advocated by the WHO as being the drug of choice for IPTp. This continued role has however been greatly overshadowed by the growing body of evidence that with an the advancement across a spectrum of severity and prevalence of SP resistance, IPTp-SP may gradually no longer present a viable option in malaria control efforts in pregnancy (164, 199, 200) despite incremental dosing showing additional benefits (3).

2.11.1 Molecular mechanisms of sulphadoxine-pyrimethamine resistance

Random genetic mutations are responsible for conferring mechanisms of antimalarial drug resistance. These mutations in eukaryotic cells occur in the order of 1 in 10^6 mitosis cycles and viable mutations are subsequently selected out under pre-existing environments (201). In addition to

mutations, gene duplication, which occur more readily than mutations, may play a significant role in the development of drug resistance.

Anti-folate resistance by *P.falciparum* is conferred by point mutations in the dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) genes. Anti-folate class of drugs, of which Sulphadoxine - Pyrimethamine, Cotrimoxazole and Chlorproguanil-Dapsone are included, exert their action through inhibiting parasite enzymes essential to parasite folate biosynthesis through decreased production of folate precursors which irreversibly inhibits the parasite life cycle (202).

Mutations in the dhfr genome have been associated with increased resistance to Pyrimethamine (203) with triple mutations conferring high resistance to Pyrimethamine in-vivo and associated with clinical treatment failure (204, 205). Aberrations in dhps accord resistance to Sulphadoxine and commonly occur as a double mutant genotype A437G, K540E. In concert with triple dhfr mutations S108N, N51I and C59R, they form a quintuple mutant haplotype that is associated with high levels of SP resistance, though not associated with a total failure of IPTp–SP (206) or exacerbation of adverse consequences of malaria in pregnancy (207). However, a sextuple mutant phenotype that incorporates an additional mutation at the dhps 581 condone (A581G mutation) exhibits high IPTp–SP failure rates (208, 209) and is a strong predictor of IPTp–SPfailure.

A previous study in the Muheza district of Tanzania demonstrated that the additional A581G mutation was strongly associated with increased parasitaemia and placental inflammation that could worsen pregnancy outcomes (16, 199). In the same area, the continued administration of IPTp–SPwas associated with an earlier time to first parasitaemia and a greater odds of severe anaemia in children born to mother who used IPTp–SP (16). These findings suggested that the additional *dhps* A581G conferred a selective advantage to parasites harboring this mutation under SP drug pressure and thus exacerbating malaria mediated placental pathology. This conclusion was however not supported by observations by a later study (210).

Irrespective of the presence of the sextuple mutation, the very existence of the quintuple mutation also renders IPTp–SP ineffective in some areas, occurring along a spectrum of ineffectiveness which is largely dependent on the prevalence of resistant haplotypes (19). A cross-sectional study in Tororo, Uganda, where the sextuple mutation is low but malaria transmission is high, showed that even more than 2 doses of IPTp–SP were not protective against individual adverse pregnancy outcomes (211). In Malawi, improvements in pregnancy outcomes have been ascribed to greater coverage of ITNS and not the continued efficacy of IPTp–SP (164). These observations may be related to the differential prevalence of the quintuple mutation compared to other areas where IPTp–SP remains effective transmission as the WHO prescribed guideline for implementation of IPT in infants (IPTi) makes the consideration that IPTi with SP should not be implemented in areas with a greater than 50% prevalence of the dhps K540E mutation as a marker for the quintuple haplotype (212).

Due to the regional differences in the prevalence of A581G associated mutations conferring super resistance and the dhps A437G mutation, which is prevalent in conferring SP resistance in west Africa (213), the continued monitoring of P. falciparum dhps A581G and K540E mutations remains essential to inform policy adaptation in respective settings (214).

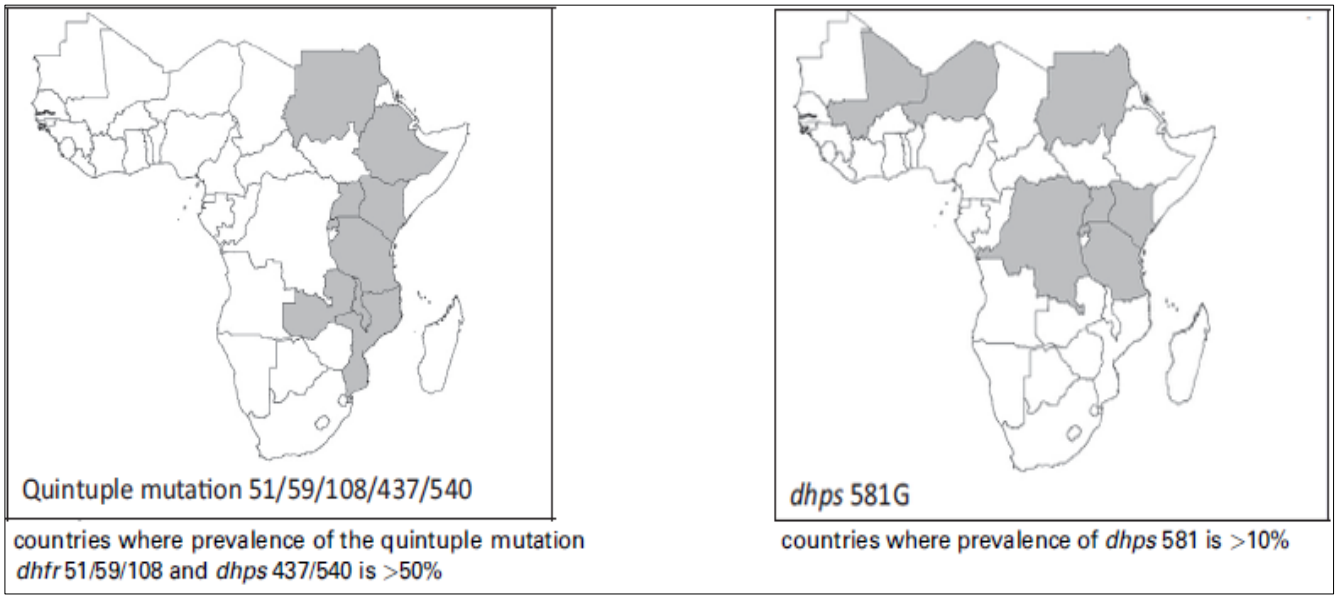
2.11.2 Drivers of drug resistance and the consequent development trajectory of SP resistance

Recent observation of the re-emergence of chloroquine naïve *P.falciparum* after an extensive period of the absence of chloroquine use strongly indicated the potentiating effect of drug pressure in driving sustained drug resistance (215-217) and igniting hope for renewed use of chloroquine for malaria treatment interventions in light of SP resistance.

The use of SP in IPTp has been associated with the competitive facilitation of drug resistant strains of *P.falciparum*. IPTp–SP was noted to select for increased fractions of parasites exhibiting A581G mutation in Tanzania (18). However, recent findings from a study in Malawi shows that the

relative reduction in drug pressure is not an adequate factor in fostering the return of SP susceptibility. Samples from patients with clinical malaria spanning periods of high, transitional and low SP use, following the switch in first line treatment policy in Malawi in 2007 to ACTs, showed an

Figure 2.2 Countries where prevalence cut-off values of major SP resistance mutations are exceeded



(Naidoo and Roper, 2013)

increase in the quintuple phenotype even during the period of reduced SP use (218). These observations may be due to sustained drug pressure from IPTp–SP (219), though minimal in comparison to population pressure exerted by use in case management, but in the absence of which may present a significant drug pressure, or cross resistance due to the extensive use of trimethoprim-sulfamethoxazole (Cotrimoxazole) in HIV management for prophylaxis against opportunistic infections (220).

Drug pressure does not appear to be the sole driver for drug resistance. Entomological studies have suggested the role of the Anopheline vector in selecting *P.falciparum* resistant phenotypes as suggested by vector control studies (221, 222). A recent study conducted in Southern

Zambia demonstrated a reduction in transmissible chloroquine resistant *P.falciparum* despite high chloroquine resistant mutants in the human population (223). This has been similarly observed for Pyrimethamine resistance (222) and associated with a restoration of SP sensitivity (224) as a result of the re-expansion of sensitive strains as in the case of the re-emergence of chloroquine sensitivity (225). Consequently, continued and aggressive vector control resulting in vector selection may facilitate the resurgence of wild type dhfr and dhps codons sensitive to SP.

2.11.3 Sulphadoxine-Pyrimethamine resistance and declining effectiveness of IPTp-SP in Malawi

Malawi was the first country to introduce IPTp-SP in 1993, and the strategy initially appeared effective. Monitoring by the Queen Elizabeth Central Hospital in Blantyre showed that between 1997 and 2006 the prevalence of placental malaria detectable by microscopy decreased five-fold from 21.2% to 4.2% in first pregnancies and ten-fold from 18.9% to 1.9% in second and subsequent pregnancies (164). At the same time there was a steady increase in ITN use from 18% to 60%; while IPTp-SP use increased from around 20% in 1997 to 78% in 2002, but dropped again to less than 50% by 2006, suggesting that the increased use of ITNs was responsible for the continued fall of placental malaria rates (164).

The same study observed that the effectiveness of IPTp-SP in Malawi appeared to reduce dramatically between 2002 and 2006, to the extent that it appeared to be no longer be protective. After 2002, women who received two or more doses of IPTp-SP were equally likely to have placental malaria, anaemia, or low birth weight babies compared to women who had not received any IPTp. During the same period, ITNs were strongly associated with higher birth weights and decreased infection rates.

These finding are consistent with an increasing impact of SP resistance on IPTp effectiveness. The degree of SP resistance in 2001-2004 was high; over 90% of parasites had the 'quintuple mutant' (*dhfr* mutations N511, C59R and S108N, and *dhps* mutations A437G and K540E) associated with SP

resistance.(226) However, previous trials have also shown that IPTp-SP provides less benefit in women who are also protected by ITNs (227); therefore the reduced efficacy of SP probably reflects the combination of high concurrent ITN use and high levels of SP resistance.

More recent observational studies of the impact of SP resistance on IPTp-effectiveness have been cause of further concern about the longevity of SP, with further evidence that at the current levels of resistance (year 2010). Preliminary analysis of the in-vivo follow-up module showed that by June 2010, 43 of 87 (49.4%) asymptomatic parasiteamic women receiving SP in their first or second pregnancy were parasiteamic again between days 7 and 42, compared to only 6 of 72 (8.3%) women in their third or subsequent pregnancies. At delivery pregnant women were equally likely to be parasitaemia when they had received the full course of SP or no SP at all (Relative risk 0.99) (Kalilani-Phiri, L and ter Kuile F, personal communication). However the risk of LBW was lower among SP recipients.

2.12 Alternative approaches to IPTp-SP in light of declining effectiveness

The problem of declining IPTp effectiveness requires urgent investigation and consideration of studies for alternative approaches to IPTp-SP. The two possible options for consideration are replacing SP alone with other drug combinations for IPTp, or alternative strategies to replace IPTp.

2.12.1 Same strategy, different drug: Alternative drugs to Sulphadoxine-Pyrimethamine for IPTp

A series of studies have evaluated several alternative drugs that could replace SP for IPTp. Any ideal drug would require to possess the characteristics outlined in the table below. The emergence of chloroquine sensitivity in Malawi (215) has inspired the consideration of chloroquine alone as a substitute for SP in IPTp (228). The results of this trial are yet to be published. Chloroquine has additionally been considered in combination with azithromycin as a viable substitute for SP in

IPTp (229, 230). However, a multicenter trial of fixed dose chloroquine-azithromycin for IPTp was terminated (231).

Azithromycin has been combined with SP to provide synergism of anti-malarial effect in IPTp as well as additional benefits of clearing reproductive tract infections associated with preterm birth and adverse pregnancy outcomes. An early trial conducted from 2003 of monthly SP with two doses of azithromycin (SPAZ) in Malawi showed a significant reduced risk of preterm delivery (RR=0.66, P = 0.01) and LBW (RR = 0.61, P = 0.02). However, this period when the trial was conducted had a low prevalence of SP resistance and as such findings may not be reproducible in the current era of near saturation of quintuple mutations (232). The postulation that SPAZ may be viable in settings of low SP resistance is further evidenced by findings from a recently published trial in Papua New Guinea (PNG) where high and super resistance phenotypes are absent (233). In this trial, SPAZ was efficacious in reducing LBW, preterm births, maternal parasitaemia and active placental malaria. A study in Ghana showed IPTp with amodiaquine or a combination of amodiaquine and SP to have comparable effects to SP (234). Findings from SP combination trials strongly suggest that any SP based combinations would likely be inappropriate in areas of high or super SP resistance.

A study in Benin showed mefloquine was more efficacious than SP for preventing placental malaria, clinical malaria, and maternal anaemia at delivery (235). However, these findings were not consistent in a trial conducted in Mozambique that found IPTp with mefloquine not to confer any additional benefit on placental infections and birth outcomes than IPTp-SP in both HIV uninfected and infected women (236, 237) and thus not supportive of a policy change.

None of the new IPTp candidates have the same favourable profile as SP alone; they are more complicated to give (azithromycin-chloroquine and amodiaquine-based regimens need to be taken once daily for three days, compared with a single dose for SP) and are not as well tolerated (such as mefloquine based regimens), which are important considerations in a strategy that targets otherwise healthy asymptomatic women.

Dihydroartemisinin–Piperaquine has also been enlisted into the band wagon of potential candidates to replace SP for IPTp. A recently concluded trial conducted in Kenya (238) has evaluated the efficacy of IPTp–DP against IPTp–SP and ISTp–DP, the results of which will only be available later in the year. A similar trial is also being conducted in Indonesia to evaluate efficacy against predominantly P.*vivax* (189).

Table 2.1 Optimal IPTp drug profile

The optimal IPTp therapy would be a combination of two molecules that:
✓ Exhibit similar time above minimum inhibitory concentrations
✓ Support a once per month dosing regimen (≤ 2 doses)
✓ Have different mode of actions to reduce resistance selection
✓ Are active against asexual & sexual stages
✓ Are not necessarily rapid acting
✓ Are either a fixed dose or loose combination
✓ Different from first line treatment for symptomatic malaria
✓ Ideally active against other treatable maternal health problems, e.g. STI/RTI, maternal-fetal transmission of infection
✓ Are safe during all the pregnancy, although pregnant women are unlikely to receive the first dose of IPTp in the first trimester
✓ Cost as little as possible per pregnancy with prices that are in line with current price estimates for artemisinin combination therapy (218)
Note: There are no new classes of medicines with plasma exposures above MIC for 28 days. Such exposure is most likely to be achieved by slow release depot formulations which would require molecules with doses of less than 10 mg/day. No such compounds currently exist.

Adapted from "Azithromycin plus chloroquine: combination therapy for protection against malaria and sexually transmitted infections in pregnancy. Chico and Chandramohan, 2011. (230)

Malaria transmission among pregnant women has reduced considerably following increased control measures including indoor residual spraying (IRS), widespread ITNs and use of ACTs in the community (ACTs kill gametocytes and can therefore help reduce transmission). This reduces the number of women at risk of malaria and the potential impact and cost-effectiveness of presumptive approaches such as IPTp. The marked reduction in exposure risk and the need for change to an

alternative drug regimen has changed the risk-benefit profile for IPTp as a strategy of choice. Furthermore the dynamic changes in malaria transmission between geographical locations negates the value of IPTp as a 'one size fits all', with alternative strategies tailored to specific transmission dynamics being required.

2.12.2 Alternative strategies to IPTp for the control of pregnancy associated malaria

In light of the diminishing prospects of IPTp being an overarching strategy for malaria in pregnancy control policy owing to the dynamic transmission landscape and lack of an ideal candidate drug, new approaches tailored to specific transmission and resistance settings are currently under consideration. In areas with high resistance but reducing transmission, interest has grown on intermittent screening and treatment as a feasible replacement to IPTp-SP. The strategy entails screening women at each antenatal visit for malaria using a malaria rapid diagnostic test and administering a treatment course with an efficacious antimalarial if the woman tests positive.

2.13 Submicroscopic parasitaemia in pregnancy: The danger that lurks in the shadows

To date the United States Food and Drug Administration (FDA) regards microscopy confirmed parasitaemia as the only approved endpoint to evaluate interventions and new diagnostics (239). Though remaining as the gold standard in many research settings, the limit of detection of malaria parasitaemia in field conditions ranges from 50 – 100 parasite per microliter of whole blood with increased rate of false negatives at low parasite densities (240). Consequently the effects of parasitaemia below the limits of detection by microscopy have been better elucidated by the use of nucleic acid amplification techniques which are highly sensitive and specific (241), providing a means of evaluating the effect of low burden parasitaemia below the threshold of conventional microscopy (242). Low density parasitaemia is common in pregnancy, with a predisposition for early pregnancy (243).

2.13.1 Pathophysiological impact of submicroscopic in pregnancy

2.13.1.1 Immune responses in pregnancy

It is well recognizes that exposure to malaria antigens is essential in the development of malaria specific immunity (244-246), however the factors determining the rate of development of this immunity are not clear, including whether in addition to exposure frequency, immunity is stimulated in a dose dependant manner to the level of parasitaemia at exposure. If and how submicroscopic parasitaemia influences the development of immunity to malaria in pregnancy remains unknown. Findings from a study in non-pregnant individuals demonstrated that low level parasitaemia inhibited dendritic cell function by induction of apoptosis (247) thus reducing malaria antigen presentation which is integral in the development of cell mediated and humoral anti-malaria immunity.

Submicroscopic parasitaemia has been associated with elevated levels of IL – 10 compared to uninfected pregnant women (248). This observation was postulated to be due to chronic low level infection detected at enrolment as similar infection at delivery did not yield any significant differences in IL – 10, possible due to the acute nature of the observed parasitaemia at that time point. Elevated IL – 10 with submicroscopic malaria has also been demonstrated in earlier work conducted in Ghana (249).

The significance of IL – 10 to malaria immunity and immunopathology appear to be dependent on the levels of IL - 10. As a member of the Th – 2 cytokine family, the effects of IL – 10 are predominantly anti-inflammatory and regulate Th – 1 responses, in particular, the regulation of TNF levels. High levels of IL – 10 reduce pro-inflammatory responses and so are protective from severe malaria disease but may be hinder cellular mediated inflammatory responses to such a degree that may allow the unchecked progression of malaria infection to severe disease. Both extremes of levels of IL – 10 have been associated with poor malaria outcomes in children (250, 251) but this remains

to be established in pregnancy. As such, the influence of submicroscopic parasitaemia on pregnancy outcomes may be dependent on the levels of IL – 10 that may be induced.

Findings from a study in Colombia has shown submicroscopic parasitaemia in the placenta to be associated with the upward regulation of pro-inflammatory cytokines, inflicted increased hypoxia and tissue damage (252). This cross sectional study at delivery compared 25 placentas with submicroscopic placental malaria against 25 sterile placenta and found elevated TNF – alpha and INF – gamma in the peripheral blood of PM + group. Higher apoptotic indexes and Fas expression were noted in the PM + group, denoting higher tissue damage, with markers of inflammation, HIF and VEGF, and hypoxia (COX – 1 and 2) being significantly higher in PM + samples. No conclusions were drawn on pregnancy outcomes from these results.

2.13.1.2 Placental integrity and pregnancy outcomes

Low density infections under the threshold of microscopy are associated with placental malaria independent of the occurrence of patent parasitaemia (243). However the association with adverse pregnancy outcomes has largely remained contradictory. In a prospective cohort study by Cohee eta al (243), submicroscopic parasitaemia was not associated with adverse birth outcomes or maternal anaemia, findings that have been corroborated by a cross sectional study in India (253) which rather found patent parasitaemia to be associated with maternal anaemia and LBW. An earlier study conducted from Cameroon likewise found microscopically detectable parasitaemia, and not submicroscopic levels, to be associated with maternal anaemia (254).

Contrary to these conclusions, a recently published longitudinal study from Benin found submicroscopic malaria to be a significant risk factor for maternal anaemia and LBW (255). An earlier study in Ghana demonstrated submicroscopic infections to be associated with raised C-reactive protein (CRP) and mild anaemia, indicating low level parasitaemia may induce a pro-inflammatory cascade (256). However, this inflammation has not been associated with adverse birth outcomes.

Rather, the presence of the parasitaemia itself has been ascribed as an independent factor contributing to LBW (113). Despite the lack of association of inflammation induced with low level parasitaemia, evidence from Colombia has implicated submicroscopic parasitaemia in causing placental villitis and intervillitis but again, this was not associated with LBW (257).

2.13.2 Impact of IPTp on submicroscopic parasitaemia

IPTp with SP clears sub patent parasitaemia although it fails to provide protection against reoccurrence of low level infection (243). The use of antimalarials for the control of malaria in pregnancy such as SP and chloroquine have been associated with submicroscopic parasitaemia (256). There is limited evidence as to the true association of IPTp and submicroscopic parasitaemia which may also be influenced by a function of the degree of maternal immunity and prevailing SP resistance.

2.14 Malaria diagnostics and their role in the control of malaria in pregnancy

The principle utilities of malaria diagnostics lie in routine case management and surveillance in control and elimination settings. The diagnostic techniques range across a variety of methods, each with their own strengths and weaknesses.

2.14.1 Clinical diagnosis

Determination of malaria disease is based on the elucidation of symptoms associated with malaria infection. However, these symptoms are usually non-specific and overlap with many other infections especially characterised by fever, vomiting and chills. Transmission intensity has great implications for the sensitivity and specificity of clinical diagnosis of malaria though the non-specific nature of malaria symptoms gives clinical diagnosis very poor specificity due to the overlap many symptoms with other infections especially those commonly characterised by fever. A study conducted in a low and unstable transmission setting in Kenya found, fever to have 88.9% sensitivity

and 15.4% specificity in children under 5 years of age, with 55.8% and 54.4% respectively in older children (258) with the addition of a second clinical criteria increasing the sensitivity but reducing the specificity. Previous studies have shown that the diagnostic performance of clinical assessment for the diagnosis of malaria varies widely over transmission intensity (259). The use of clinical diagnosis for treatment decisions remains largely unreliable and leads to inefficient syndromic management (260, 261).

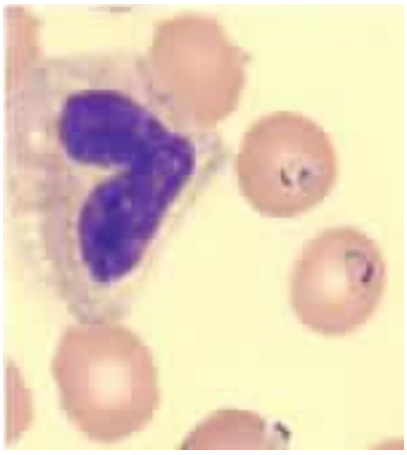
2.14.2 Light microscopy with Giemsa stain

Light microscopy examination of Geimsa stained blood films has been the mainstay of malaria diagnosis for clinical care and disease surveillance, and has been regarded as the gold standard against which new malaria diagnostic tests are evaluated. Though operational costs are low, making it suitable for resource constrained environments, with added advantages of being able to provide speciation and quantification of parasitaemia, the method suffers several draw backs in the need for robust technical expertise which may not always be readily available (262), is time consuming (263) making it less ideal in settings of high disease burden, and requires vigorous equipment maintenance, quality control and quality assurance.

Under ideal conditions, light microscopy examination of thick blood film is able to detect parasitaemia as low as 4 parasite per microliter (264), with this detection threshold being higher in field settings up to 100 parasites per microliter. Thick blood film is more sensitive to detection of malaria parasitaemia than thin blood films, with the latter usually used for species identification.

Preparation of a blood film for examination under light microscopy involves spreading a sample of the blood as a thick or thin film, staining with a Romanovsky stain, commonly Geimsa, and examining the slide with a 100 x objective under oil immersion. Thick film microscopy has been shown to underestimate parasite densities likely as a result of staining process conferring them with a reduced sensitivity compared to PCR in terms of detection and quantification (265).

Figure 2.3 Examination of malaria parasitaemia under a light microscope



Adapted from "Malaria diagnosis". Centers for Disease Control and Prevention, 2015. (266)

False positive results with light microscopy arise commonly due to artifact inclusion due to poor preparation as well as poor technical expertise by the microscopist (267). Conversely, the false negative rate increases with declining parasite density (268). This underscores the need for greater expertise and time to diagnose malaria by microscopy to ensure a reduction in missing infections. Errors in species identification are largely under reported which is reflected in the under reporting of mixed infections, especially of *P.falciparum* and *P.vivax*, where differentiation between the species is not done (267, 269). Misclassification of *P.knowlesi* as P.*malariae* have been observed (270, 271), further stressing the weakness of microscopy especially where this distinction would be critical such as in south-east Asia where *P.knowlesi* malaria is fast spreading. Under reporting of the quality of microscopy has been noted even in published manuscripts where microscopy is regarded as the reference standard (168).

Methods to further enhance microscopic detection of parasitaemia have been developed. The quantitative buffy coat (QBC) technique involves staining the parasite DNA with fluorescent dyes such as acridine orange, with reading conducted by epi-fluorescent microscopy (272). These enhanced microscopy techniques have demonstrated higher sensitivity than conventional Geimsa

microscopy (273) even at low parasitaemia (274) but specificity is compromised due to staining of white cell DNA. In addition, speciation and quantification of parasitaemia is not feasible. Notwithstanding, this technique has been strongly considered as a feasible replacement to Geimsa microscopy for qualification of malaria infection in the routine case management diagnosis of malaria (273) and has become a growing diagnostic method of choice for field epidemiology studies due to the added advantage of sensitivity at low parasitaemia levels.

2.14.3 Immunochromatographic diagnostic techniques

Malaria antigen based tests were initially pioneered by the development of immunoflorescent antibody and enzyme linked immunosorbent assays (IFA and ELISA respectively) with commercial application remaining scarce and use largely relegated to research purposes (275-277).

Immunochromatographic assays, commonly known as malaria rapid diagnostic tests (mRDTs) were developed based on bound antibodies detecting specific malaria antigens such as histidine rich protein 2 (HRP – 2), plasmodium lactate dehydrogenase (pLDH) or aldolase which would elicit a chromatographic response, in the form of a colored line, if the specific antigen is present in the blood sample (278). The WHO sets a required sensitivity for rapid diagnostic tests used for active case management of malaria of at least 95% at a parasite density of 100 parasites per microliter (279, 280).

The efficiency of RDTs is largely dependent on the intensity of transmission that dictates the mean parasite density associated with clinical symptoms as well as the prevalence of infections that has an impact on the sensitivity of the test (281, 282). Evidence from performance analysis in children show that RDTs demonstrate a drastic decline in sensitivity at parasite densities below 200 parasites/ μ L (283). Target antigen expression is crucial in determining the calibre of RDTs appropriate in particular settings. In areas in Latin America and India, the existence of HRP2-deleted

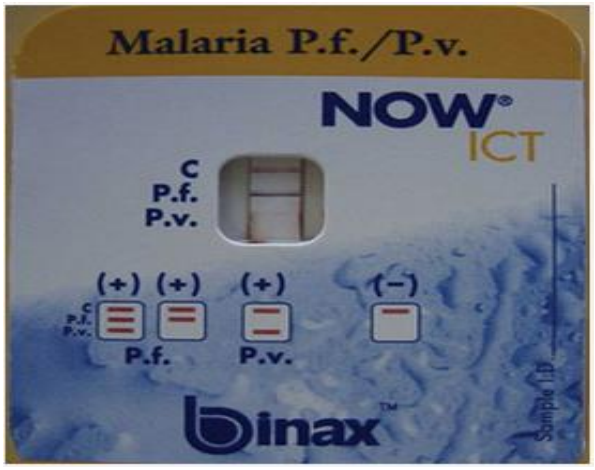
strains of Plasmodium have been identified (284-286) and as such HRP2 based RDT would have dismal performance in these areas and would largely be inappropriate. Polymorphism of HRP2 and 3 surface antigens may impact negatively on HRP-based RDTs in areas with HRP-expressing parasites (287), though a previous study that conducted a pooled analysis of HRP2 antigens of *P.falciparum* isolates from 38 countries did not demonstrate the sequence variation as a cause for sensitivity variation. However, performance of HRP-2 based RDTs may be further compromised by high titres of HRP-2 antibodies (288).

Further complicating the reliability of malaria RDTs is the observation of cross reactivity of autoimmune factors and bacterial antigens leading to false positives and consequentially an overestimation of parasitaemia and treatment. Iqbal and colleagues demonstrated that *P.falciparum* HRP2 tests gave a false positive result in 26% of patients with rheumatoid factor but were aparasitaemic by microscopy and PCR (289). In tropical settings where both malaria and bacterial infections, individually or as co-infections, abound, false positivity due to cross reactivity possesses a considerable concern. Non-Typhiodal Salmonella bacteraemia has been associated with increased RDT positivity in the presence of a negative gold standard malaria test (290) and a recent case report documented a positive malaria rapid diagnostic tests with Salmonella typhi without evidence of co-malaria infection (291).

A plethora of studies have been conducted evaluating the performance of several rapid malaria diagnostic tests in field settings and controlled laboratory environments. The evidence is well summed in a systematic review and meta-analysis by Abba et al in 2011 and a later publication by Maltha et al in 2013. Abba and colleagues categorized RDTs based on antigen combinations incorporated in the test for diagnosis of malaria with classes 1 – 3 having HRP-2 alone or in combination with other antigens and types 4 and 5 being anchored by pLDH (168). Of 74 studies incorporated in the analysis, 73% were types 1 – 3. The pooled performance estimates of the HRP-2 based tests demonstrated high sensitivity and specificity of 95.0 % (95% C.I, 93.5% - 96.2%) and

95.2% (95% C.I, 93.4 % - 99.4 %). The meta-analytical averages for type 4 and 5 tests had lower sensitivity but higher specificity, at 93.2% (95% C.I, 88.0 % – 96.2%) and 98.5% (95% C.I, 96.7 % - 99.4%) respectively. Conclusively, HRP-2 assays would miss less cases but pLDH tests would correctly diagnose more cases.

Figure 2.4 A positive rapid malaria diagnostic test



Adapted from "Malaria Diagnosis – Rapid Diagnostic Test". Centers for Disease Control and Prevention, 2014. (292)

The majority of RDTs on the market are tailored toward the diagnosis of *P.falciparum* and *P.vivax*, demonstrating poor detection of *P.ovale* and *P.malariae* (293, 294). RDTs targeting conventional malaria antigens expressed by *P.falciparum* and *P.vivax* have been utilized for the detection of the newest identified species of Plasmodium causing malaria in humans, *P.knowlesi.* Some RDTs have been able to diagnose *P.knowlesi* infection (295) but with sensitivities markedly below the WHO recommended cut off of 95%. RDTs with greater diagnostic reliability, preferably

based on an antibody specific for *P.knowlesi*, present a pressing niche in product development in light of the proliferation of *P.knowlesi* malaria disease in south-east Asia (296, 297).

In a review by Maltha et al (298), the reliability of RDTs was concluded to be mainly compromised by poor detection at low parasitaemia due to design limitations. Paradoxically, tests were also susceptible to the hook/prozone effect, precipitating false negative results in instances of extremely high parasitaemia. Deficiencies in the expression of target antigens such as HRP-2 or the cross reactivity with other antigens has rendered test performance vulnerable to either poor or exaggerated performance indicators respectively. Other factors such as end user errors and product packaging were also faulted in contributing to poor field performance of RDTs.

The WHO, in conjunction with the Centers for Disease Control and Prevention (CDC), TDR and FIND, conduct routine rounds of test performance for commercially available RDTs manufactured under ISO 13485:2003 quality system standard. The most recent round of RDT performance testing, Round 5, was conducted in 2013 (299). The panel detection score (PDS) is the main test score used to evaluate RDT performance. Of note is the great emphasis that has been placed on greater expectation of robust test performance at lower parasite concentrations, reflecting the growing need for more sensitive diagnostics at lower parasite densities (< 200 parasites/ μ L) that may still render clinically significant disease owing to reductions in parasite transmission in light of current eradication efforts in many endemic countries.

Results from the fifth WHO round of RDT performance evaluations showed that HRP2 based tests had the highest detection rates with pLDH only tests registering some of the poorest results. This was particularly evident at parasite densities of under 200 parasites/ μ L, with all evaluated tests having high detection at parasite densities greater than 2,000 parasites/ μ L. The proportion of tests that achieved a PDS greater than 75% at 200 parasites/ μ L were 78.6% and 42.4% for *P.falciparum* and *P.vivax* respectively. This highlights the marked deficit and the unmet need for

reliable tests for *P.vivax* (300), the implications of which could result in difficult administration of parasitaemia confirmed treatment guidelines in areas endemic to vivax malaria. Most of the evaluated tests demonstrated heat stability, an important factor in the confidence in test results as they are mostly used in tropical climates which register high temperatures, and are easy to use with the majority of tests being in cassette form.

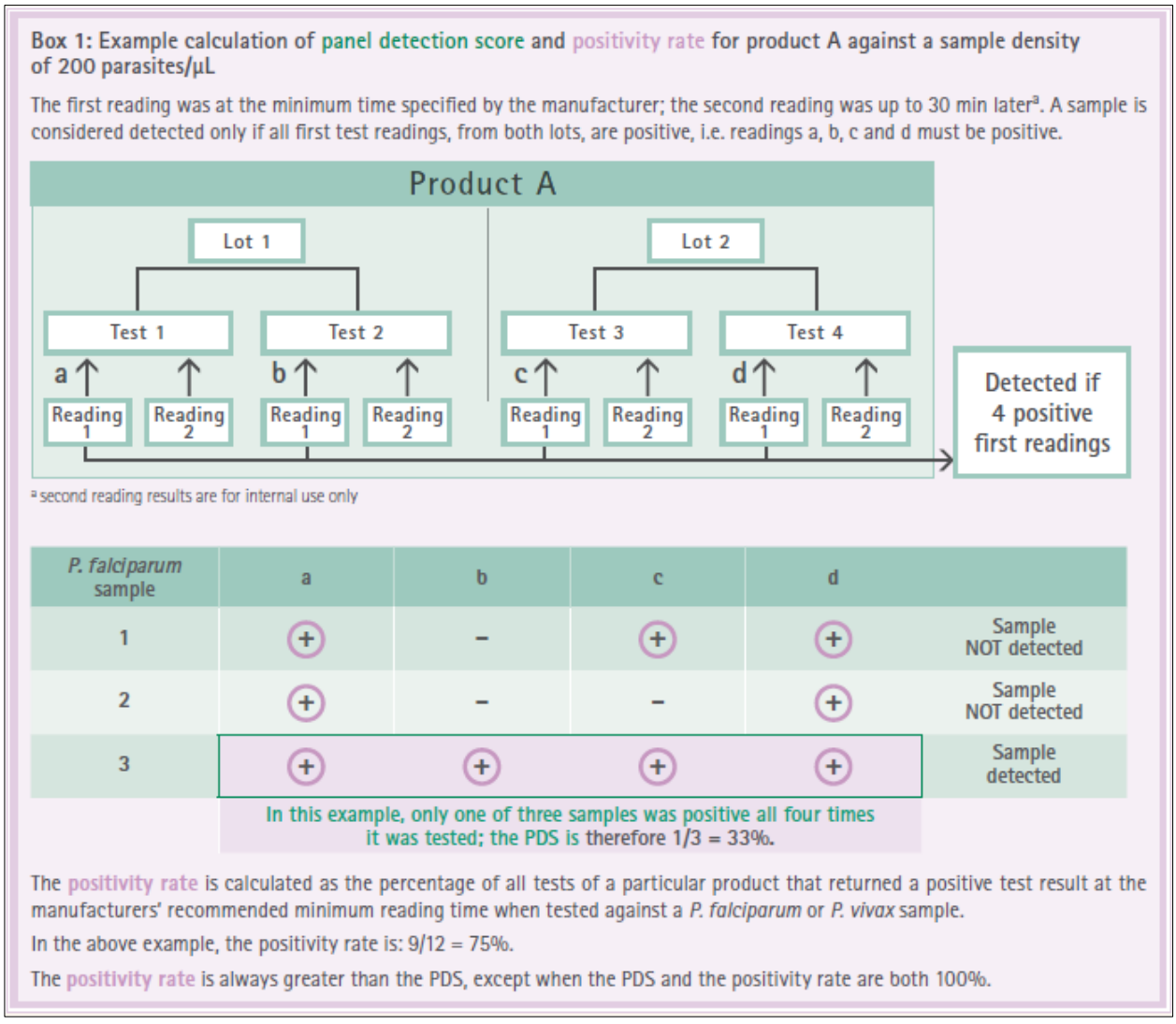
2.14.4 Molecular techniques

Molecular techniques based on the amplification of parasite DNA to detect malaria infection are more sensitive than RDTs and microscopy (301, 302), especially in low transmission settings and have gained increasing popularity in epidemiological and drug efficacy studies (303). Since the advent of these techniques in the 1980's and 1990's (304), several variants of these molecular techniques have been developed with varying reliabilities.

Molecular diagnostic methods for malaria include mass spectrophotometry, automated blood cell counters, flow cytometry assays, microarrays, loop-mediated isothermal amplification (LAMP) and polymerase chain reaction (PCR) assays. Of all, PCR assays have emerged as the most useful in malaria epidemiology registering the highest sensitivity and specificity even at low parasite densities. PCR is able to detect parasitaemia as low as 1 – 5 parasite per microliter, conduct species characterization and more importantly, evaluate for markers of drug resistance. The importance of these utilities for practical malaria epidemiology have become evident as PCR is the only currently reliable diagnostic modality for the fast emerging *P.knowlesi* malaria complex (305, 306) and the detection of mutations conferring resistance to anti-malarials such as artemisinin, SP and chloroquine (307-310) .Several variations of PCR methods have been developed such as nested PCR, real-time PCR, reverse transcriptase PCR and polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP). PCR remains limited to research purposes and high performance diagnostic laboratories due to the extensive demand on expertise, time and equipment.

As such PCR has not yet been modified for field use though this would be an ideal development for the optimization of malaria diagnosis for routine treatment and surveillance. LAMP and microarrays have however demonstrated significant potential for field deployment (311) and may provide more sensitive diagnostic modalities than current thick smear microscopy and RDTs.

Figure 2.5 Calculation of PDS for the evaluation of test performance



Adapted from "Summary results of WHO product testing of malaria RDTs: Round 1-5 (2008-2013)". World Health Organisation. 2013. (299)

2.14.5 Placental histology

To date there are no reliable diagnostics for the detection of placental malaria in-vivo. The growing need for in-vivo applicable diagnostics would greatly revolutionize the diagnosis and management of malaria in pregnancy and has been identified as an unmet need in malaria control efforts (300) and for the improvement of maternal and child health in malaria endemic settings.

Sequestration of erythrocytes infected by *P.falciparum* in the placenta is currently only diagnosable "post-mortem" by histological examination of placental sections after delivery and insults already rendered. This is too late for any meaningful interventions to be implemented to improve pregnancy outcomes that may have been adversely affected by placental malaria. As such, the histological diagnosis of placental infection has been relegated to research purposes as an endpoint of malaria in pregnancy studies.

Previously, placental malaria on histological examination of placental sections has been characterised by sequestered infected erythrocytes only (acute infection), infected erythrocytes with co-existing hemozoin in fibrin lattice (chronic infection) and hemozoin only in macrophages and / or fibrin (past infection) (92). Active malaria infection was associated with basal membrane thickening, fibrinoid necrosis and syncytial knotting. A semi-quantitative grading system has been previously developed by Muehlenbachs and colleagues (93) in which a novel grading scheme was utilized to quantify inflammation and hemozoin deposition in placental malaria and evaluate the associations with pregnancy outcomes. Scores were demonstrated to be associated with decreased birthweight in samples from both high and low transmission settings, indicating the versatility of the method across divergent transmission intensities. The utility of the method was underlined as enabling the comparison and standardization of results between studies where placental malaria is an endpoint.

The importance of diagnostics for in-vivo diagnosis of active placental malaria are underscored by histological findings of the placental architecture in active placental malaria described by histological examination. In a study of the morphometric and histological changes

observed in *P.falciparum* and P.*vivax* infections, Chaikitgosiyakul and colleagues demonstrated that the area for nutritional delivery is significantly reduced in active placental malaria due to a reduced number, size and vascularity of villi (92, 312). This distorted architecture has been observed to be reversible after efficacious treatment, implying that the detrimental effects of active placental infection can be reversed insitu if placental malaria can be reliably diagnosed and appropriately treated prior to delivery.

2.14.6 Measures of diagnostic test performance

2.14.6.1 The notion of the Gold Standard

The continued consideration of light microscopy as the gold standard for malaria diagnosis has been subject to heavy criticism in light of more sensitive diagnostic tests such as nucleic acid amplification based tests especially with the increased unreliability in expanding low transmission settings. A systematic review that employed a statistical analysis avoiding the dependence of a gold standard against which other tests would be evaluated found increased reliability of non-microscopy tests for the diagnosis of malaria (274).

2.14.6.2 Evaluation of test performance

The performance of a diagnostic tests lies in its ability to discriminate between diseased and healthy conditions and correctly detect the disease of interest. Diagnostics in screening environments have to demonstrate the ability to detect pre-disease conditions or markers that are harbingers of disease in order for interventions to be administered to prevent the full realization of the disease state. Several parameters are employed to assess the reliability of diagnostic tests for their chosen scope of utility. The rapid progress in the development of diagnostic tests presents a revolutionary prospect for personalized medicine and as such, the importance of the reliability of diagnostic tests will be imperative (313).

2.14.6.2.1 Sensitivity, specificity, positive predictive values and negative predictive values

A comparison between an index test and a gold standard tests is the commonest used method to assess performance of an index test. This is accomplished by deriving parameters indicative of the index test performance from a 2 x 2 table:

Figure 2.6 2x2 table for calculation of measures of diagnostic performance

	Gold standard disease present	Gold standard disease absent	
Test positive	True positives (TP)	False positives (FP)	Total test positives:
	a	b	a+b
Test negative	False negative (FN)	True negatives (TN)	Total test negatives:
	c	d	c+d
	Total diseased:	Total normal:	Total population:
	a+c	b+d	a+b+c+d

Adapted from "Understanding and using sensitivity, specificity and predictive values". Parikh et al, 2008. (314)

The sensitivity of the index test is its ability to correctly classify diseased individuals. This is derived as a proportion of the (true) positives by the gold standard identified by the index test (a/a+c). The specificity is the index test's ability to correctly discriminate un-diseased individuals. This is expressed as the proportion of (true) negatives by the gold standard identified by the index tests (d/b+d). The ideal test would have 100% sensitivity and 100% specificity but realistically, there is a trade-off between sensitivity and specificity. It is recommended that estimates for the precision of the index test be reported for studies evaluating performance of diagnostic tests (315). The 95% confidence interval limits for sensitivity and specificity can be calculated (315) but cannot exceed 100% (316).

The positive predictive value (PPV) of the index test is the efficiency of the test in having correctly identified diseased individuals who have been diagnosed by the test as having the disease. This is calculated as the probability of a patient having the disease when tested positive by the index

test and is given by (a/a+b). The negative predictive value is the efficiency by which the test correctly identifies an un-diseased individual and is given by the probability of a patient being negative when tested negative by the test (d/c+d). Both the PPV and NPV are a function of the prevalence of the disease in the population. The prevalence of a disease in the population has a positive association with PPV and a negative association with NPV.

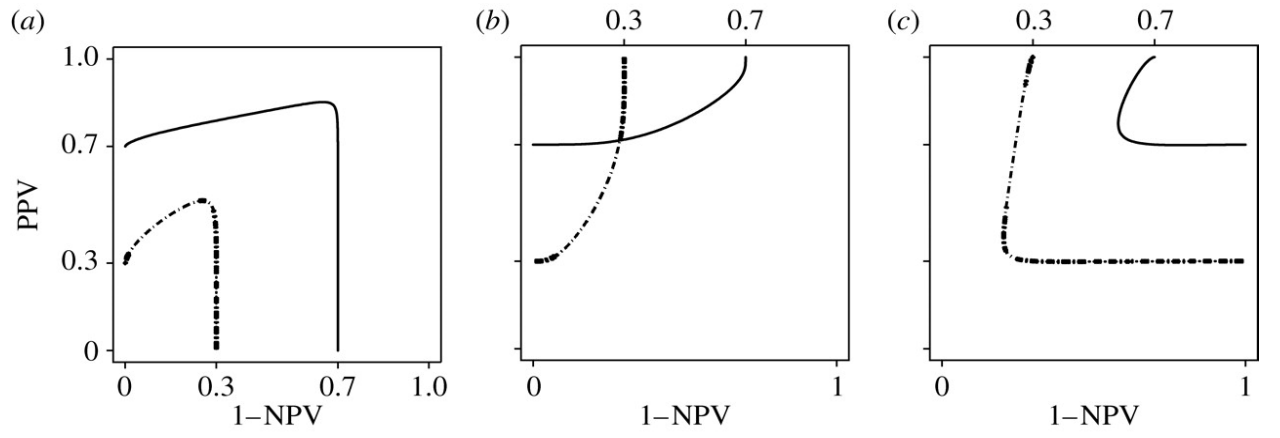
The likelihood ratios for positive results (LR+) and negative results (LR-) are additional performance measures of the efficiency of the index test in a specified population. The LR+ is dependent on the sensitivity of the index test and the proportion of false positives, and is given by:

sensitivity/ (100% – specificity)

A value greater than 1 indicates the test is associated with the disease and describes the strength of the association of the test with actually having the disease compare not having the disease. Values greater than 10 are indicative of a high performance diagnostic. Conversely, the LR- is dependent on the specificity and is given by:

(100% – sensitivity)/ specificity

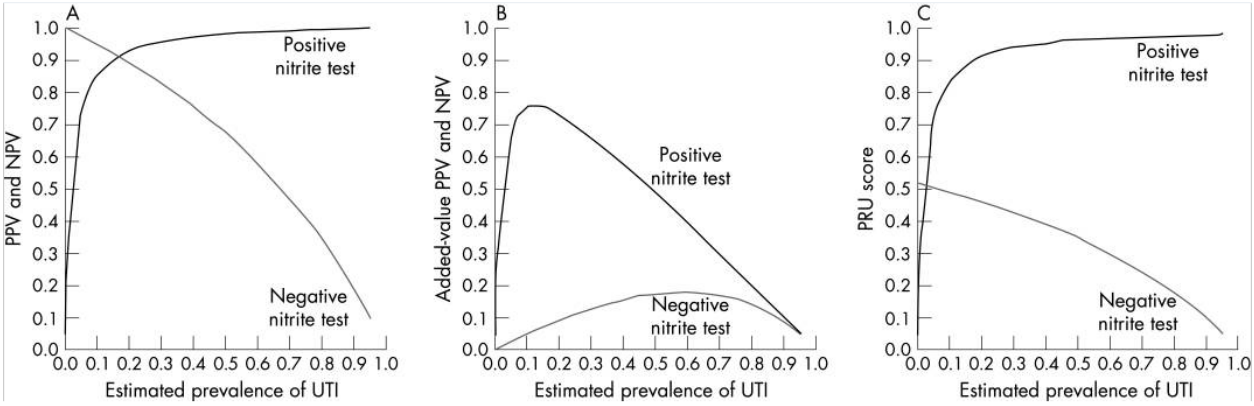
Figure 2.7 PROC curve as a function of disease prevalence



Predictive curves with a=0.8. (a) b=0.7, (b) b=1, (c) b=1.5. Solid line, high prevalence (p=0.7); dot-dashed line, low prevalence (p=0.3).

Adapted from "The predictive receiver operating characteristic curve for the joint assessment of the positive and negative predictive values. Shiu and Gatsonis, 2008. (317)

Figure 2.8 PPV and NPV at different levels of urinary tract infection prevalence in children



Plots of (A) the positive and negative predictive values (PPV and NPV), (B) the value added PPV and NPV, and (C) the proportionate reduction in uncertainty scores (PRU) for diagnosing a urinary tract infection (UTI) in a child as a result of carrying out a urinary nitrite stick test, according to the estimated likely prevalence of UTI given that individual's clinical circumstances.

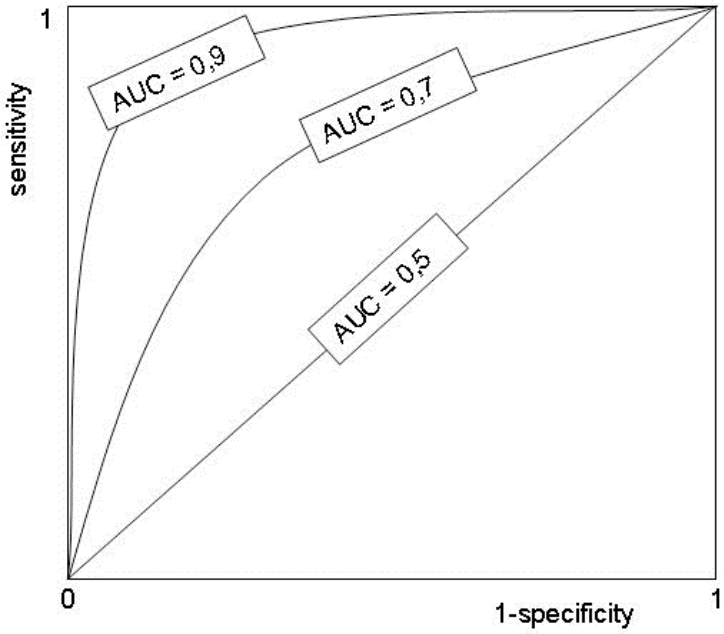
Adapted from "Quantifying how tests reduce diagnostic uncertainty". Coulthard, 2007. (318)

An LR- of less than 1 shows that the test is associated with the absence of the disease. The value describes the probability of a diseased individual being tested negative (319). Values less than 0.1 are indicative of a good diagnostic test. As the LRs are derived from parameters not dependant on the prevalence of disease, they can be compared across studies provided the disease definition is constant between studies (320). Ancillary measures of diagnostic accuracy include ROC curves and Area Under the Curve (AUC), Diagnostic Odds Ratio (DOR), Diagnostic effectiveness and Youden's index.

2.14.6.2.2 Ancillary measures of diagnostic accuracy

The ROC curve is a plot of the values of the diagnostic sensitivity and specificity pairs with 1 – specificity against sensitivity. The discriminative power of the test is evident by the shape of the curve as well as the area under the curve. The greater the area under the curve is by virtue of the curve approximating the upper left hand corner of the graph, the greater the diagnostic accuracy of the test.

Figure 2.9 The ROC curve and AUC



Adapted from "Measures of diagnostic accuracy: basic definitions". Šimundić, 2008. (320)

The AUC is useful in comparison of two or more tests to assess which is more appropriate diagnostic as interpretation of the sensitivity and specificity of individual tests is not possible. The comparison of the AUC between tests requires statistical inference evaluating the difference between AUCs.

The diagnostic odds ratio (DOR) is a ratio of the odds of a test indicating a true positive against the odds of the test indicating a false positive. It is given by:

$$\mathbf{(TP/FN) / (FP/TN) = (a/c) / (b/d) = (ad/bc)}$$

It does not rely on the prevalence of the disease but is rather dependent on the sensitivity and specificity of the test.

Table 2.2 Relationship between the area under the ROC curve and diagnostic accuracy

Area	Diagnostic accuracy
0.9 - 1.0	Excellent
0.8 - 0.9	Very good
0.7 - 0.8	Good
0.6 - 0.7	Sufficient
0.5 - 0.6	Bad
< 0.5	Test not useful

Adapted from "Measures of diagnostic accuracy: basic definitions". Šimundić, 2008. (320)

The diagnostic accuracy defines the percentage of index test results that agree with the gold standard test. This is a measure of the effectiveness of the index test and is given by:

$$\mathbf{(TP + TN) / N = (a + d)/ (a + b + c + d)}$$

The DOR is inversely related to the prevalence of the disease meaning the test will give more correctly classified individuals as the prevalence of the disease decreases (320). It is advised that the DOR be interpreted in the context of other measures of accuracy such as the predictive values.

The Youden's index is a whole number derived from the sensitivity and specificity as:

$$\mathbf{(sensitivity + specificity) - 1}$$

The closer to 1 the index score is, the better the test. However, the index is unreliable in inferring the diagnostic accuracy between tests as tests may have the same score but different measures of sensitivity and specificity (320) and thus us not a reliable measure to discriminate the accuracy between tests.

2.14.6.3 Reporting of diagnostic accuracy studies: The STARD Statement

The Standards for the Reporting of Diagnostic Accuracy studies (STARD) were developed in 2003 to address the less than optimal reporting of studies evaluating the diagnostic accuracy of tests (321). The statement comprises a checklist of 25 items to ensure all relevant information is reported

in the manuscript (Annex 1). Much is still expected in the adoption of the STARD protocol (322) though no revision of the statement have been undertaken since first publication.

2.15 **Disease screening**

The concept of disease screening as a strategy for the reduction of disease associated morbidity and mortality is based on the potential benefits of early detection of asymptomatic preclinical disease or disease precursors and administration of an effective intervention. Accordingly, the ten point Wilson-Jungner criteria for appraising the validity of a screening programme is essential in considering the viability of a screening strategy (323) though recent alternatives have been proposed after a review of the Wilson-Jungner criteria in the current scientific dispensation (324-327).

The following criteria are considered in evaluating if a disease screening strategy is warranted (323):

1. The condition sought should be an important health problem
2. There should be an accepted treatment for patients with recognised disease
3. Facilities for diagnosis and treatment should be available
4. There should be a recognisable latent or early symptomatic stage
5. There should be a suitable test or examination
6. The test should be acceptable to the population
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood
8. There should be an agreed policy whom to treat as a patient
9. The cost of case finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible medical expenditure as a whole
10. Case finding should be a continuing process and not a “once and for all” project

2.16 **Innovation for the control of malaria in pregnancy**

The extensive burden of malaria infection in pregnancy amongst the world's poorest populations, coupled with the myriad of challenges faced in its control begs for a renewed drive for innovative strategies. With the faltering of IPTp-SP as an effective strategy in *P.falciparum* endemic regions due to expanding SP resistance, potential strategies are faced with the challenge of achieving optimal target population coverage whilst ensuring safe and efficacious dose delivery for optimal protection and the prevention of the emergence of drug resistance. It would be expected that new strategies would work in synergy with LLITNs for the prevention of infections, reducing the burden of maternal, fetal and newborn morbidity and mortality. Significant gaps in knowledge remain in the safety of dihydroartemisinin-piperaquine in pregnancy and the role of low density infections not detected by diagnostic tests in routine clinical practice in low resource settings on pregnancy outcomes. Furthermore, though extensively used in routine clinical care for symptomatic malaria infection, the efficiency of RDTs in diagnosing asymptomatic infection, many largely persisting as low density infections, presents a significant challenge to the reliability of RDTs as part of control strategies for the control of malaria in pregnancy. This trail and its subsequent analyses therefore provide the opportunity evaluate targeted treatment of asymptomatic infection in pregnancy in a setting of declining transmission, the significance of low density infections on pregnancy outcomes and the efficiency of RDTs as a screening tool in asymptomatic malaria infection.

3 CHAPTER THREE: OVERVIEW OF STUDY DESIGN AND METHODS

3.1 Background

In the context of the existing body of evidence for the decreasing efficacy of IPTp–SP and the need for alternative drug and strategy interventions, this study was designed to evaluate the efficacy of 3 or 4 courses of ISTp – DP in comparison to a similar number of courses with IPTp–SP. The study was designed before the revised 2012 guidelines for IPTp–SP by the WHO.

The primary objectives of the trial were:

1. To determine if ISTp-DP compared with IPTp-SP in the second and third trimesters of pregnancy is associated with at least a 25% reduction in adverse birth outcome as composite endpoint of low birth weight, preterm birth and small for gestational age in paucigravidae.
2. To determine if ISTp-DP compared with IPTp-SP in the second and third trimesters of pregnancy is associated with at least a 50% reduction in placental malaria in multigravidae.

The sections that follow describe the specific design considerations for the trial as well as study procedures and statistical methods of analysis employed. A brief overview of statistical methods of the derived analyses from the main trial in the two subsequent analyses is presented, with further descriptions of specific methods provided in the respective chapters.

3.2 Trial design

3.2.1 Overview

This was an open-label, two-arm, individually randomized controlled superiority trial conducted at three sites in southern Malawi with high levels of SP resistance and ITN coverage. A stratified enrolment design by gravidity was employed with one strata for primi and secundigravidae (G1+2) and one for multigravidae (G3+). The study required 1665 women overall, comprising of 1155 paucigravidae and 500 multigravidae.

Participants were randomly allocated to receive either at least three doses of IPTp with SP or at least three scheduled screenings with an RDT and treatment with DHA-PQ if they are RDT-positive. All

participants were given an insecticide-treated bed net. Women enrolled in the trial made at least three scheduled visits to the clinic, including the enrolment visit, spread over the second and third trimesters at least four weeks apart, approximately mirroring the appointment schedule for 'focussed antenatal care' in Malawi which consists of four scheduled visits including one in the first trimester. Women who were enrolled by 25 weeks of gestation were asked to attend 4 scheduled visits in the 2nd and 3rd trimester with those enrolled after 25 weeks being requested to attend 3 scheduled visits. Dating ultrasound scans were performed to assess gestational age.

Other than the investigational interventions, all participants received the standard antenatal care according to local policy, including standard clinical examinations, tests (section 3.8.1.2) and treatment as required (section 3.7.3). Administration of dihydroartemisinin-piperaquine (DHA-PQ) was done under direct supervision, with follow-up visit on day 3 and 14 post the first dose to assess tolerability and safety. Women were encouraged to attend the study clinic for assessment if they felt unwell during the study period, and to deliver in the maternity wards of the study sites to facilitate collection of delivery outcome data. In the event that they delivered outside of the study facilities, women were encouraged to notify staff as soon as possible, preferably within 24 hours but with a leeway of up to 7 days post-delivery. Maternal infant pairs were seen at approximately seven days and six weeks after delivery, to assess the postnatal health of both mother and infant before fully exiting the study.

3.2.2 Study design

3.2.2.1 Design considerations

3.2.2.1.1 Superiority design

It was anticipated that ISTp would be more complex to implement and potentially more expensive than IPTp and, unlike with IPTp, sub-patent infections would not be prevented or suppressed, thus concomitant use of ITNs was important. Countries with moderate transmission

would only consider switching to a new strategy if it was clearly superior to the current policy of IPTp-SP, either because of SP failure or because SP would become potentially harmful.

3.2.2.1.2 Efficacy versus effectiveness

The trial was designed primarily to assess the efficacy of ISTp when implemented as intended. This proof of concept was needed as a logical step before further assessments of effectiveness in practice were undertaken, as it was not yet established in this context. In addition, as any effect will usually tend to be greater under conditions of optimal implementation, the sample size required would be smaller for an efficacy trial than for an effectiveness trial. Therefore efforts were made to maximise participant adherence to the study protocol, including active follow-up of participants who missed scheduled appointments. Data was collected to assess the tolerability of the intervention and levels of adherence under the trial conditions.

3.2.2.1.3 Open label design

Participants were informed of the intervention they are assigned to as the processes for IPTp and ISTp were visibly different and thus blinding would not have been possible. Blinding was however undertaken for diagnostic tests performed by laboratory personnel.

3.2.2.1.4 Exclusion of HIV-positive women

Women who were HIV seropositive were not included as all HIV-positive women would have been eligible to receive co-trimoxazole prophylaxis of HIV-related infections, as recommended by WHO. Co-trimoxazole itself provides some protection from malaria infection, and SP is contra-indicated in women receiving co-trimoxazole. It would therefore not have been possible to include HIV-positive women in the current trial, as they could not be included in the IPTp-SP arm.

With an estimated HIV prevalence of around 12% in pregnancy, it would be important to evaluate the potential effectiveness of ISTp in HIV-positive women, but this would require a different

trial answering a different question i.e. comparing co-trimoxazole prophylaxis alone with co-trimoxazole prophylaxis plus ISTp.

3.2.2.1.5 Intervention schedules

The study intervention was delivered at intervals of four to eight weeks and as far as possible corresponding to the usual schedule of 'focused' antenatal care visits during the second and third trimesters of pregnancy which are scheduled at approximately 24-28 weeks, 30-32 weeks, and 34-36 weeks. Women attending between 16 and 23 weeks received an additional RDT screening or SP dose, as with IPTp this is what tends to happen in practice in Malawi. The last intervention in both arms occurred at approximately 34 to 37 weeks of gestation, to ensure clearance of the placenta before delivery. As per usual standard of care, women who had not delivered by 40 weeks were seen weekly up to 42 weeks gestation and if not yet delivered, would have labor induced as part of the clinical obstetric care in accordance with clinical guidelines. These visit were not classed as study visits, and had no influence on the administration of study interventions or procedures.

The regimen for IPTp in Malawi used to be at least two doses of SP during the second and third trimesters of pregnancy. However, Malawi switched to a more pragmatic approach providing SP at each scheduled visit as part of focussed antenatal care, with many women now receiving three or four doses of SP prior to the 2012 revised guidelines by the WHO. As the trial was designed to determine the superiority of a new, more expensive and complex intervention (ISTp) to current practice (IPTp), it was imperative that the current intervention be implemented in its most effective form possible.

3.2.2.1.6 Inclusion of women with uncomplicated malaria at enrolment

Women with symptoms of malaria at enrolment were not excluded, but were randomized to the study groups, and treated with the case-management drug for the respective trial arm. Women

in the IPTp–SP group were treated with Artemether-Lumefantrine (CoArtem) and did not receive SP that month due to Lumefantrine being anticipated to provide post-treatment prophylaxis comparable with SP (3 to 4 weeks). SP was then be provided at the next scheduled visit. Women randomized to the ISTp arm received DHA-PQ as the treatment drug. This was done to reflect standards of practice of treatment of uncomplicated malaria in the current dispensation of IPTp-SP and the proposed ISTp-DP strategy within routine healthcare provision at the antenatal clinic. DHA-PQ and CoArtem have comparable efficacy in treating malaria.

3.2.2.1.7 Additional blood tests not part of the point of care testing and treatment

In addition to the use of RDTs in the ISTP group, all participants in both arms had blood samples taken at enrolment and during each scheduled visit for peripheral malaria using standard microscopy of blood smears and PCR. The results of these tests were not available until the end of the trial and were not used to inform the care of the women. In this way, information on incidence of malaria infection was collected without contaminating the intervention in either arm. A blood sample was taken at the last visit in the third trimester for haemoglobin measurement and a second HIV screening test in line with current WHO guidelines for repeat HIV testing in the third trimester (328).

3.2.2.1.8 Stratified sampling of women with different gravidities

Based on systematic review of previous trials with IPTp-SP and ITNs (14, 159), it was anticipated that most or all of the impact on birth outcomes and morbidity would be in women in their first and second pregnancies. However, the impact on placental malaria infection was expected to occur across all pregnant women, including those in their third and subsequent pregnancies. Placental malaria and malaria at term are recognised to be important endpoints in themselves as they have been found to be risk factors for malaria, anaemia and other infections in infants up to the

age of two years, independent of the effect of malaria on gestational age low birth weight (96, 329). This effect appears to be particularly evidence in multigravidae (123).

Furthermore, as IPTp is currently recommended for, and provided to women of all gravidities, and it was anticipated that any alternative strategies to IPTp may also be provided to all pregnant women, if any potential benefit is identified. The trial therefore focused on morbidity endpoints in women in their first and second pregnancies, and on malaria infection at term and delivery in women in their third to fifth pregnancies (active or past infection).

3.2.2.1.9 Use of a composite birth outcome as the primary trial endpoint

In women in the first and second pregnancies, the study aimed primarily to assess the effect of the intervention on birth outcomes. A composite primary outcome of SGA, low birth weight or pre-term birth was used because more births would fall into one of these categories than into a single category. In addition, malaria during pregnancy is associated with increased risk of all these three measures, including an increase in the risk of SGA in children born at term and with birth weight > 2500 g. Therefore a smaller sample size would be required to detect a significant difference between study intervention arms than if a single outcome were used. SGA is a risk factor of neonatal and infant mortality, also in those both at term or with normal birthweights (330).

Early neonatal deaths were not included within the composite endpoint measure because the aetiological fraction of deaths due to malaria was anticipated to be small; most early postnatal deaths result from events occurring during delivery (e.g. prolonged labour).

3.3 Outcome measures

3.3.1 Primary outcome

In paucigravidae the primary outcome was a composite endpoint of adverse birth outcomes, defined as any of:

- Small for gestational age defined as a binary outcome of <10th percentile of fetal weight for attained gestational age
- Preterm birth (spontaneous birth before 37 weeks gestation)
- Low-birth-weight (birth weight under 2,500 grams)

For women in their third to fifth pregnancies, malaria infection at term and delivery were the primary endpoint. These were defined as evidence of current or recent infection assessed at delivery by placental histopathology, a positive malaria RDT or PCR.

3.3.2 Secondary outcomes

3.3.2.1 Secondary efficacy outcomes

1. Placental malaria (any species):
    a. Past infection detected by histopathology
    b. Active infection detected by:
        ii. Histopathology
        iii. Microscopy
        iv. Rapid diagnostic test
        v. Polymerase chain reaction (PCR)
2. Maternal malaria infection (peripheral blood) at delivery, detected by:
    a. Microscopy
    b. RDT
    c. PCR
3. Peripheral malaria infection during pregnancy detected by:
    a. Microscopy
    b. PCR
4. Birth weight

a. Mean birth weight (grams)

b. Low birth weight (<2,500 grams)

5. Gestational age

    a. Mean gestational age at birth (grams)

    b. Pre-term birth (<37 weeks)

6. Small for gestational age

7. Maternal haemoglobin and anaemia:

    a. At delivery

        i. Mean maternal haemoglobin (g/dL)

        ii. Anaemia (Hb ≤ 11 g/dL)

        iii. Moderate to severe anaemia (Hb ≤ 8g/dL)

    b. During third trimester

        i. Mean maternal haemoglobin (g/dL)

        ii. Anaemia (Hb ≤ 11 g/dL)

        iii. Moderate to severe anaemia (Hb ≤ 8g/dL)

8. Miscarriage (loss of foetus before 28 weeks gestation)

9. Stillbirth (birth at 28 weeks or later showing no signs of life)

10. Composite endpoint of the primary endpoint plus fetal loss (miscarriage or stillbirths)

11. Infant death

    a. Perinatal death (stillbirth or death within 7 days of birth)

    b. Neonatal death (death within 28 days of birth)

12. Malaria infection of the newborn, detected by analysis of umbilical cord blood with:

    a. RDT

    b. Microscopy

    c. PCR

13. Foetal haemoglobin and anaemia by sampling of umbilical cord blood at birth:
    a. Mean foetal haemoglobin (g/dL)
    b. Foetal anaemia (Hb ≤ 12.5 g/dL)
    c. Moderate to severe foetal anaemia
14. Incidence of documented clinical malaria episodes during the second and third trimesters of pregnancy (history of fever in last 24 hours and documented malaria microscopy or RDT positive)
15. Presence of any evidence of malaria infection at term (last antenatal visit), identified through microscopy or PCR, or at delivery, identified through peripheral and placental RDT, microscopy or PCR, or placental histopathology (active or past infection).
16. Incidence of other illness episodes apparent at scheduled antenatal clinic visits or resulting in unscheduled clinic visits
17. Incidence and prevalence of clinical malaria in infants by seven days and six to eight weeks determined by:
    a. RDT
    b. Microscopy
    c. PCR
18. Prevalence of symptomatic infant anaemia at seven days and six to eight weeks
    a. Anaemia
    b. Moderate to severe anaemia
19. Incidence of other illness episodes in the infants, apparent at scheduled postnatal clinic visits or resulting in unscheduled postnatal clinic visits

3.3.2.2 Safety outcomes

1. Severe cutaneous skin reaction in the mothers within 30 days of drug intake

2. Other serious adverse events in the mothers
3. Congenital malformations identified by six weeks after birth
4. Neonatal jaundice within 24 hours and at seven days
5. Laboratory test results outside of normal range

3.3.2.3 Tolerability outcomes

1. Non-serious adverse events in the mothers
2. Adherence to study medication

3.4 Study sites

The study was conducted in three research centers in southern Malawi with moderate to high malaria transmission (331). The first site, located 10 kilometers south of Blantyre in the Shire Highlands, the study was conducted at Mpemba health centers which serves a predominantly rural populace and characterised by mild temperature and humidity.

Further south, approximately 30 and 50 kilometers from Blantyre respectively, the study was conducted at Madziabango health center, under the administrative jurisdiction of Blantyre district though close to the Shire valley, and Chikwawa District Hospital located in the Shire Valley, both sites having characteristic high temperatures and humidity due to the topographic influences of the Shire valley.

3.5 Sample size

The trial utilised a stratified design with one strata for primigravidae and secundigravidae and one strata for multigravidae. It was designed to detect a 25% reduction (RR 0.75) in composite adverse birth outcomes (SGA, low birth weight and pre-term birth) in women in their first and second pregnancies with an 90% power and a 2-sided significance level of 0.05, if the rate of placental malaria in the control group was similar to that found ongoing observational studies of women

receiving routine care in the same area of Malawi. The proposed difference was based on four previous trials of IPTp which jointly reduced low birth weight by 29%.(14). Thus the efficacy of ISTp was expected to be similar to the efficacy of IPTp before the emergence of widespread SP resistance. In an ongoing IPT-mon study at the time, the incidence of SGA, low birth weight or preterm birth was 40.3% (N=494) (Kalilani, personal communication). Therefore the study was designed to detect a 25% difference between 40.3% in the control (IPTp) group and 30.2% in the intervention (ISTp) group. The study would still have 80% power (instead of 90%) to detect a 25% reduction if the risk were at least 33.3% instead of 40.3%.

For this primary endpoint, 491 participants were required in each arm or 982 in total. To allow for a 15% loss to follow-up, 1155 women in their first and second pregnancies were recruited. The sample size had an 81% power to detect a 25% or greater reduction in placental malaria (active or past infection), with a significance level of 0.05. If a power of 80% were used, the study would detect a difference of 21.8% or greater in the primary endpoint.

The trial was also designed to detect a 50% reduction in past or present placental malaria infection at delivery in multigravidae, based on previous studies of IPTp,(14) assessed by placental histopathology and RDT. The rate in women in Malawi who were taking IPTp-SP was estimated from an observational IPTp–SP study at 20.5% (Kalilani, personal communication). A reduction by 50% to 10.25% would require 213 women per arm or 426 in total. To allow for a 15% loss to follow-up a total of 500 multigravidae were to be recruited. The total sample size was therefore 1655.

3.6 Enrolment and Randomization

3.6.1 Screening and eligibility assessment

Potential participants were screened for eligibility when attending their first routine antenatal appointment. If they met the inclusion criteria they were offered enrolment following

informed consent. Women who did not meet the inclusion criteria, or who did not wish to participate in the trial received the usual antenatal care, including the standard IPTp-SP schedule.

Eligibility screening was conducted by clinic nurses and midwives and supported by study staff. Screening data was recorded in a log kept in the investigators site file. One screening log was kept for each site. The record reported how many women attended their first antenatal visit over the enrolment period, how many were potentially eligible, how many were enrolled into the study and reasons for non-eligibility of those not enrolled. It was used to ensure that women were selected for the study without bias. Each screened woman was assigned a screening registration number in sequential order by the study clinic. Screened attendees who did not meet the eligibility criteria for the trial were considered as screening failures. Screening registration numbers were not re-used. Women were allowed to enter the study at a later date upon meeting the eligibility criteria if she was previously unable to do so at the prior visit.

Gestational age was initially estimated using a combination of fundal height as measured by the midwife and last menstrual period (LMP) dates provided by the woman, where available. Women attending antenatal care for the first time after 28 weeks gestation were excluded from the trial. Women attending before 16 weeks gestation were screened for inclusion, given information about the trial and enrolled when they next attend between 16 and 28 weeks gestation. Women who were eligible and expressed an interest in the trial were subsequently enrolled and received an ultrasound scan using the SonoSite™ S180 machine (SonoSite, Inc, Bothell, Washington, USA) to accurately assess the gestational age of the pregnancy and identify multiple gestations. Alternatively the scan was done at a rescheduled appointment shortly after enrolment if it could not be performed on the day of enrolment. Ultrasound scans were conducted by a trained clinician, who was also the study investigator as well as the study nurses who had also received training by a clinical radiologist. Any women identified as having multiple gestations at ultrasound scan were excluded from the study.

Women already known to be HIV-positive were automatically excluded from the study. All women were offered HIV counselling and testing using rapid diagnostic tests, and if they refused or were found to be positive, were be excluded from the trial. All potentially eligible women were tested for anaemia. If they are found to be severely anaemic (Hb ≤ 7 g/dL) they were excluded from the trial and referred to the nearest hospital for blood transfusion and appropriate treatment. A woman who was successfully treated for severe anaemia and attended the clinic again before 28 weeks gestation was deemed eligible for participation in the trial provided that she met all other inclusion criteria. Women with less severe anaemia were be included in the study and their anaemia treated by treating any cause identified and with ferrous sulphate 200-400mg twice daily for a month. A venous blood sample was used for both routine and study-specific tests. As venous sampling would not normally be included as part of care, consent for screening procedures that required venous blood samples was obtained before the blood draw.

Women were asked whether they planned to travel or migrate outside of the study area during the follow-up period. Those who planned to so were excluded. Women who were later found to be ineligible after they had signed the informed consent and initiated study procedures were excluded from the trial as screening failures immediately, but continued to receive all study-specific benefits and standard care.

3.6.2 Informed consent procedures

A general introduction to the study and invitation to participate was provided by a study nurse during the antenatal talks at each clinic over the enrolment period. More specific study information was provided in a consulting room (Annex 1). Participants were given leeway to consider the information including consultation with their spouses or immediate family, if required. Spousal involvement was encouraged in the woman's participation in the study from as early as the consenting stage.

There were three elements to consent for involvement (Annex 2); consent for eligibility screening; consent for inclusion in the trial; and consent for storage of blood samples. Consent for screening was sought from all women who appeared potentially eligible at the pre-screening stage, and was specific to procedures that are not normally undertaken as part of routine care. These included a 5ml venous blood draw taken before enrolment, which would be used to assess the woman for anaemia, and, if the test had not already been undertaken, HIV and syphilis, as well as for study-specific tests if the women were found eligible.

Informed consent for inclusion in the trial was sought immediately before enrolment after 16 weeks gestation. Women who made first contact before 16 weeks were potentially eligible received all relevant information at this time, and were asked for consent to be screened, but were not asked for their formal written consent for participation in the trial until their next attendance after 16 weeks.

Each potential participant was given full and adequate oral and written information about the study, including all the known risks and any potential benefits, in their own local language. It was clearly stated that the participant would be free to withdraw from the study at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal. Participants were given the opportunity to ask questions and allowed time to consider the information provided.

Informed consent was documented on a written consent form signed by the participant and the person who conducted the informed consent discussion. If the participant was unable to write her signature then a thumbprint was used. If the subject was unable to read the information herself, full and comprehensive verbal information was communicated in the presence of an impartial witness. The witness signed the consent form to attest that information in the consent form was accurately explained and apparently understood by the participant, and that informed consent was freely given.

Participants were asked for additional consent for the transport of blood sample outside of the country and the storage of blood samples for future research. Women who did not consent to the storage and transport of blood, or who withdraw their consent, were still allowed to participate in the trial. In accordance with the laws of Malawi, a pregnant women aged 16 years or older is considered emancipated (332) and would be able to sign the consent form for themselves; no parental consent was required for emancipated minors. Either carbon copies or otherwise two identical consent forms were used per woman; one signed or thumb-printed consent form was kept on file by the study team and one given to the participant.

3.6.3 Treatment allocation (Randomization)

Two randomization sequences were computer-generated by the study statistician at Liverpool School of Tropical Medicine, one for women in their first and second pregnancies and another for multigravidae. Variable block randomization was used, stratified by study site to ensure an equal proportion of participants in each intervention group from each site. The variable length of each block ensured that allocation concealment was fully maintained and allocation well distributed over the seasons. The allocation ratio for the two study arms was 1:1.

Recruitment was 'competitive' between study sites, meaning that the number of participants recruited at each site differed depending on the respective rates of enrolment. A sufficient number of randomization codes were generated for each site to allow for this.

Site specific randomization sequences stratified by gravidity were used to assign women to study arms. An independent person prepared opaque envelopes which were numbered sequentially with the allocated group code and details for that number inside. For each newly enrolled participant, an envelope from the correct sequence (according to gravidity group) was opened sequentially to identify the group that they are allocated to, thus concealing the upcoming allocation from the participants, clinic staff and study staff. The number on the envelope represented the study number

allocated to that participant and will became their unique identifier in the trial. Once assigned, a study number could not be used again, even if a participant discontinued. In the event a study number was allocated incorrectly, it was not reassigned and randomization continued with the next sequential number.

3.7 Administered treatments

3.7.1 The study interventions

Participants received one of the following two interventions during the second and third trimesters of pregnancy; provided at each of the scheduled visits as directly observed treatment (DOT):

IPTp-SP Group: Treatment with a treatment dose of three tablets of sulphadoxine-pyrimethamine, each containing sulphadoxine (500 mg) and pyrimethamine (25 mg). This is the standard and only drug of choice for IPTp in Africa.

ISTp-DP Group: Screening for malaria was done using a combined Histidine – Rich Protein 2 (HRP-2)/ plasmodium Lactate Dehydrogenase (pLDH) (*P. falciparum/* pan-malaria) rapid diagnostic test (First Response® Malaria pLDH/HRP2 Combo Test, target antigen pLDH (pan); HRP2; Premier Medical Corporation Ltd, USA). Each tablet of Dihydroartemisinin-piperaquine came as a fixed dose combination of 40 mg Dihydroartemisinin and 320 mg Piperaquine. Women were treated with dihydroartemisinin-piperaquine (Sigma Tau) with 2-2.3 mg/kg/day dihydroartemisinin and 16 – 18.3mg/kg/day Piperaquine for 3 days if RDT-positive. The daily number of tablets administered depended on the weight of the woman and was given to the nearest half tablet per recommendations by the manufacturer. All half tablets that remained after each clinic visit in the morning were stored in the drug inventory for drug accountability and were not administered to patients at subsequent antenatal clinic visits.

All investigational medicinal products were sourced from GMP approved manufacturers. Upon receipt from the suppliers, all the drugs were stored at the clinical trials pharmacy unit with restricted access and temperature controlled environment at less than 30 degrees Celsius for long term storage. Drugs were dispensed to the trial sites in cooler boxes at a maintained temperature of less than 40 degrees Celsius where upon reaching the trial sites, the drugs were stored in temperature monitored secured storage units and dispensed to patients in accordance with the treatment protocol. Routine monitoring of drug inventory was undertaken at both the central trial pharmacy and trial sites to reconcile drug stocks.

3.7.2 Case-management of clinical malaria

Women in either group who attended scheduled study appointments with fever and parasitaemia confirmed by RDT were treated for malaria. In the case of women in the IPTp-SP group, case-management treatment with Artemether - Lumefantrine (Coartem®, Novartis) in a fixed dose combination of 20mg Artemether and 120mf Lumefantrine with 4 tablets per dose, 8 hours apart between the first two doses then 12 hours for the remaining 4 doses, was given in place of the scheduled dose of SP. Women in the ISTp group were treated with DHA-PQ as direct observed treatment (DOT). DOT was not employed for women in the IPTp–SP arm who were being treated for symptomatic malaria.

3.7.3 Other treatment and concomitant medication

3.7.3.1 Treatments as part of routine care

All participants were offered routine antenatal care according to local policy and the principles of 'focussed antenatal care', including the provision of insecticide-treated bed nets (ITNs) free of charge. In addition, the routine antenatal care package in Malawi includes blood screening at the first appointment for syphilis, anaemia and HIV, and the provision of appropriate treatment for these conditions. Women diagnosed with symptomatic anaemia were treated in accordance to the

severity. In cases of symptomatic mild or moderate anaemia, women were prescribed an additional 200mg of elemental iron as ferrous sulphate or if unavailable, ferrous folate, for one month. In the event of severe anaemia (haemoglobin <7g/dl), admission and blood transfusion was instituted at the referral health facility, followed by an additional 200mg of elemental iron once a day for one month after discharge. In the event a woman was diagnosed with syphilis, she was requested to present the following day with her partner where after both then received 2.4 million units of benzathine penicillin G intramuscularly once a week for 3 weeks. If the spouse did not present for treatment, the women was still treated. Women diagnosed before or during the trial with HIV-infection were excluded or withdrawn from the trial respectively and offered co-trimoxazole prophylaxis and counselling services in preparation for initiation of antiretroviral treatment in accordance to national guidelines and WHO recommendations. Blood screening for anaemia and HIV were also be repeated in the third trimester, as per standard of care in accordance to policy in Malawi (anaemia) and per new WHO guidelines (HIV).

As part of their antenatal care participants were given iron (200mg/day) and folic acid (0.4 mg to 0.6 mg / day) supplements as well as tetanus vaccination as applicable. Presumptive treatment for Helminthic infestations was with an Albendazole 400 mg single dose for hookworm, trichuriasis and ascariasis.

3.7.3.2 Treatments at unscheduled study visits

Participants were encouraged to attend the study clinics if they felt unwell at any point outside the scheduled antenatal visit. All concomitant medications prescribed to participants during the study, or reported as used by the participants were recorded. Women diagnosed with symptomatic uncomplicated malaria were treated as described above. Rescue treatment with oral quinine, quinine sulfate dihydrate 600 mg every 8 hours for 7 days, was used in the event that a woman became symptomatic and parasiteamic again within 4 weeks. The exception was women in

the ISTp group who were attending their first or other routine appointments, would receive DHA-PQ if RDT-positive, with CoArtem reserved as second line therapy and oral quinine as third line. Women with severe malaria were admitted to hospital for treatment with intravenous quinine.

3.7.3.3 Prohibited treatments

Prohibited medication included antimalarial drugs not prescribed within the trial protocol, and other drugs with antimalarial properties including co-trimoxazole. Participants who took prohibited medications remained in the trial but were excluded from the per-protocol analysis.

3.8 Use of long lasting insecticide treated bed nets (LLITNs)

All eligible participants were provided with a long lasting insecticide treated bed net at booking and were encouraged to consistently and correctly use the bed nets, not only for the duration of the trial, but also after their participation. Monitoring of LLITN use during the trial was based on self-reported compliance by the participant at scheduled study visits. These reports of LLITN use were not corroborated through observation at the house hold level.

3.9 Schedule of follow-up visits and procedures

A summary of the schedule of visits is provided in Figure 3.1 and Table 3.1 below. The detailed description of the timing and conduct of study visits is provided as follows:

3.9.1 Antenatal booking visit

The first study visit immediately followed eligibility screening if a woman made contact with the study team between 16 and 28 weeks gestation. If a woman made her first contact before 16 weeks gestation, the first study visit was conducted at their next appointment between 16 and 28

weeks gestation. Informed consent was obtained at the first contact visit after completion of eligibility screening and before enrolment.

3.9.1.1 Enrolment

After informed consent had been granted participants were randomly allocated to receive either IPTp or ISTp as described earlier. Participants were informed of study intervention they have been allocated to.

3.9.1.2 Baseline assessment

A baseline assessment was conducted for each participant comprising demographic information, socioeconomic information, insecticide treated net (ITN) and indoor residual spraying use, medical and obstetric history, and clinical assessment. Any relevant information already collected during the screening process or at previous antenatal appointments for the same pregnancy was copied from the antenatal cards to avoid unnecessary duplication of work.

Study-relevant information recorded at the first visit included age; area of residence; prior use of ITNs; number of previous known pregnancies and number of previous births. Clinical assessment included height and weight, fundal height, reported date of last menstrual period and estimated gestation.

A 5 ml venous blood sample was taken and used for both routine and study-specific testing. This was obtained after consent for eligibility screening has been sought but before consent for enrolment in the trial. Women who had not been tested for HIV in their current pregnancy were tested and all women were assessed for anaemia and haemoglobin levels. Women not previously tested for syphilis in this pregnancy were tested and treated if positive. The same blood sample was used for l be prepared for later study-specific testing for malaria (blood smear microscopy and PCR) and other assays for related studies.

3.9.1.3 Study intervention

The first dose of IPTp-SP or RDT screening for ISTp-DP was administered to asymptomatic women at this visit. If women had symptomatic malaria, they were treated as described.

3.9.1.4 Routine antenatal care and treatment of illness

Routine antenatal care and treatment of any illness identified at this visit were provided. This included provision of a suitable LLITN and advice on sleeping under the net for the duration of the pregnancy.

3.9.1.5 Prior morbidities and medications in the current pregnancy

All participants were asked about any symptoms or illnesses they have had in the last month, and any medications taken.

3.9.2 Second and third subsequent visits

A second clinic visits was scheduled between four and six weeks after the preceding visit with an additional third visit only for participants who were enrolled in the trial at between 16 and 23 weeks gestation.

3.9.2.1 Study interventions and care

A subsequent dose of IPTp-SP or RDT screening for ISTp-DP was administered to asymptomatic women at this visit. If women had symptomatic malaria, they were treated as earlier described in section 3.7.2. Participants were asked about any symptoms or illnesses they have had since the last antenatal visit and any medications taken that had not been prescribed from the study clinic.

All consenting participants had a venous blood sample taken, prepared and stored for later malaria testing using standard microscopy of blood smears and PCR of dried blood spots. Testing was done with the technicians blinded to the participant's intervention arm.

Figure 3.1 Flow chart of participant study visits

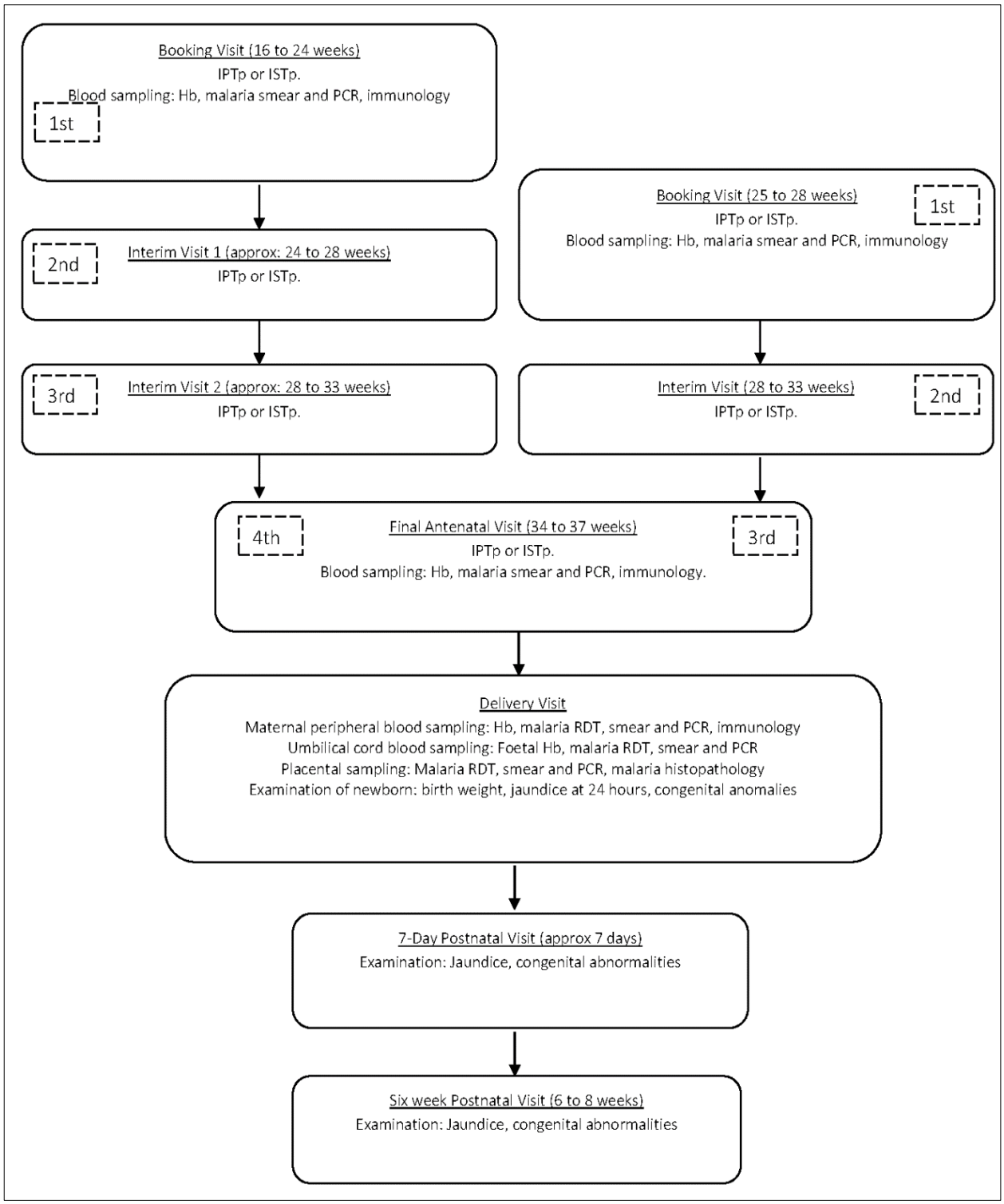

Booking Visit (16 to 24 weeks)
IPTp or ISTp.
Blood sampling: Hb, malaria smear and PCR, immunology
1st
Booking Visit (25 to 28 weeks)
IPTp or ISTp.
Blood sampling: Hb, malaria smear and PCR, immunology
1st
2nd
Interim Visit 1 (approx: 24 to 28 weeks)
IPTp or ISTp.
3rd
Interim Visit 2 (approx: 28 to 33 weeks)
IPTp or ISTp.
Interim Visit (28 to 33 weeks)
IPTp or ISTp.
2nd
4th
Final Antenatal Visit (34 to 37 weeks)
IPTp or ISTp.
Blood sampling: Hb, malaria smear and PCR, immunology.
3rd
Delivery Visit
Maternal peripheral blood sampling: Hb, malaria RDT, smear and PCR, immunology
Umbilical cord blood sampling: Foetal Hb, malaria RDT, smear and PCR
Placental sampling: Malaria RDT, smear and PCR, malaria histopathology
Examination of newborn: birth weight, jaundice at 24 hours, congenital anomalies
7-Day Postnatal Visit (approx 7 days)
Examination: Jaundice, congenital abnormalities
Six week Postnatal Visit (6 to 8 weeks)
Examination: Jaundice, congenital abnormalities

Table 3.1 Table summary of study visits

	Booking visit (16 to 28 weeks)	**Interim visit** (approx 24 -33 weeks)	**Additional Interim visit** (women enrolled	**Final antenatal visit** (34 to 36 weeks)	**Telephone call for RDT+ women ISTp+ 2 days**	**Home visit for RDT+ women ISTp+ 2 days**	**Clinic visit for RDT+ women ISTp+ 14 days**	**Delivery**	**7-day postnatal visit** (7 days)	**6-week postnatal visit** (6 -8 weeks)
Actions										
Eligibility confirmed	X									
Consent	X									
Randomization	X									
IPTp or ISTp	X	X	X	X						
Measures										
Adherence monitoring					*Sub-sample	*Sub-sample				
Tolerability/ adverse events		X	X	X	*Sub-sample	*Sub-sample				
Blood biochemistry							*Sub-sample			
Full blood count							*Sub-sample			
Blood drug levels							*Sub-sample			
Immunology	X			X				X		
Peripheral malaria microscopy and PCR	X			X				X		
Peripheral malaria RDT								X		
Hb and anaemia**	X**			X**						
Placental microscopy, RDT and PCR								X		
Placental histopathology								X		
Umbilical cord blood RDT, microscopy and PCR								X		
Birth weight								X		
Gestational age								X		
Congenital anomalies								X	X	X
Neonatal jaundice								X	X	
Blood sample for storage	X			X				X		
HIV**				X**						
Blood markers of malaria immunity and iron deficiency	X							X		

*Women in the ISTp-DP group who were RDT-positive and treated with DHA-PQ

**Routine care

3.9.3 Final antenatal visit

A final third or fourth clinic visit was scheduled between 34 and 37 weeks gestation, at least four weeks after the previous visit.

3.9.3.1 Study interventions and care

In addition to procedures conducted during the interim visits, all women had their haemoglobin levels tested as point of care. Women found to be anaemic (Hb ≤ 11 µg/ L) were treated as appropriate. In addition, women were re-tested for HIV, as recommended in the latest WHO guidelines.(328) as well as having urine checked for protein and glucose.

3.9.4 Day 3 and 14 post-treatment visit (RDT-positive women treated with DHA-PQ only)

Women treated with DHA-PQ were asked to return to the clinic 3 and 14 days after the first dose was administered for assessment of safety. In case of the occurrence of a severe adverse event, a blood sample was taken to later have drug levels assayed. A venous blood sample was at day 14 taken for assessment of full blood count (haemoglobin, white blood cell count, platelets); and biochemistry (bilirubin, alanine aminotransferase and creatine).

3.9.5 Unscheduled visits during pregnancy

Participants were encouraged to visit the clinic in the event they felt unwell between scheduled appointments. Participants who presented between appointments were examined by study staff. Presenting symptoms, axillary temperature and blood pressure were recorded. A venous blood sample was taken for Hb measurement and malaria RDT and thick and thin microscopy smears. Women presenting with serious adverse events possibly associated with DHA-PQ had a 2 ml venous blood sample taken to measure blood drug levels. Any illness was treated as appropriate and according to standard local care.

3.9.6 Delivery visit

Women were encouraged to deliver on the maternity wards of the participating clinics, in which case they would have delivery assisted by a skilled birth attendant. A member of the study team would visit them on the ward and perform relevant examinations shortly after delivery. Women who delivered at home were identified and traced using a network of community health workers and were visited by a member of the study staff or encouraged to report to the research clinic within 48 hours, to collect, as far as possible, the same information as women who had delivered at the health facility.

3.9.6.1 Peripheral blood sampling

A 5 ml venous blood sample was taken from the mother and tested for malaria using RDT, standard microscopy and PCR; and tested for Hb concentration using hemocue. Women found to have malaria infection by RDT or microscopy, or anaemia (Hb ≤ 11 g/L) were treated in accordance with standard practice.

3.9.6.2 Placental sampling

A placental blood sample was collected from the delivered placenta through an incision on the maternal side and collecting the pooled blood. This sample was tested for malaria using RDT, standard microscopy and PCR. A 2cm x 2cm x 1cm specimen of placental tissue was taken from the maternal side for histopathology testing for current and past malarial infection. In addition, a roll of the amnion and chorion were taken for assessment of infection in these two areas.

3.9.6.3 Umbilical cord sampling

An umbilical cord blood sample from the placental side of the cord was taken and tested for the fetal haemoglobin level as well as malaria infection using RDT, standard microscopy of blood smear and PCR. If the pLDH line of the RDT was positive for malaria, microscopy would be prioritised

for within the next 24 hours to ascertain infection. In the event of microscopically proven parasitaemia, the baby was referred to the hospital for treatment of congenital malaria.

3.9.6.4 Examination of the baby

The baby was weighed using accurate digital scales and examined for vital status, presence of any significant congenital anomalies, and gestational age at birth using the Ballard's score. After 24 hours, the baby was re-examined for the presence of jaundice.

3.9.7 Postnatal visits

Maternal – neonate pairs were seen at the clinic around seven days after birth, if possible to correspond with the baby's first vaccination visit. The baby was examined for the presence of jaundice and for any congenital anomalies that may have been missed at delivery. Babies with symptoms of malaria had blood samples taken from a heel prick for testing by RDT and treatment provided based on the RDT results. Standard postnatal advice and healthcare was given as needed for mother and baby. Women who did not attend the appointment were traced at home. Any infant deaths occurring before the visit were recorded and a verbal autopsy used to ascertain the likely cause of death. Participants and their babies were again seen between six and eight weeks after birth, at their baby's second vaccination visit with similar procedures being conducted as at the week 1 post-natal visit. In addition, participants were also asked about their use of healthcare for the baby since birth.

Women were advised to present to the clinic in the event that themselves or their baby became unwell during the postnatal follow-up period. Any illness were treated as appropriate and according to standard local care.

3.10 Clinical tests and Laboratory methods

The following diagnostic procedures were performed on blood and urine samples as stipulated in the respective visit procedures. All rapid diagnostic test were conducted in line with the manufacturer's recommendations.

3.10.1 Clinical tests

3.10.1.1 Rapid diagnostic tests for malaria

The First Response® Combo Malaria Ag (pLDH/HRP2) (Premier Medical Corporation, Ltd, India) test was used for assessment of parasitaemia for point of care and screening. After confirmation of the participant's identification number, the test kit was labeled with the participants ID and visit date. Blood obtained in accordance with procedures relevant to the visit was then used in order of specifications by the manufacturer and results interpreted as described in the Figure 3.2.

3.10.1.2 Rapid diagnostic tests for syphilis and HIV

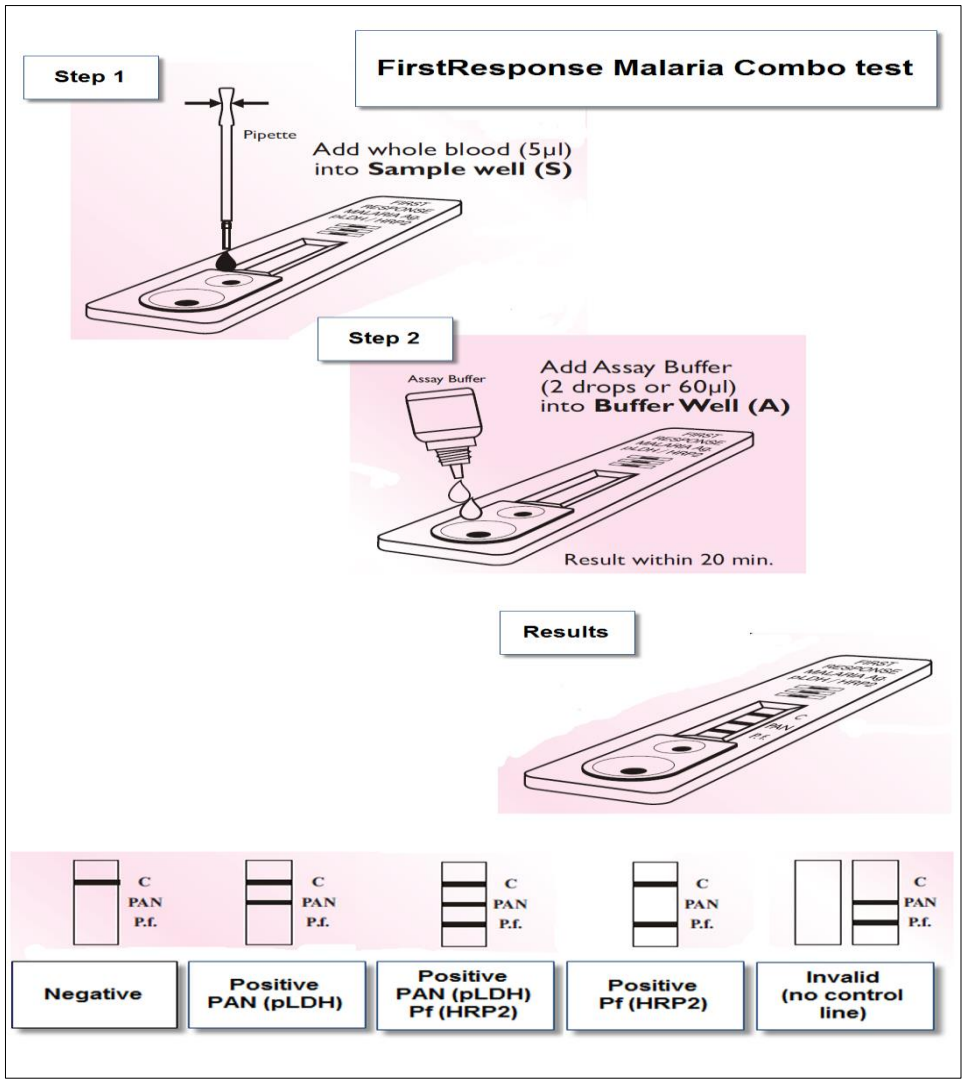
The SD BIOLINE Syphilis 3.0 test solid phase immunochromatographic assay for the qualitative detection of antibodies of all isotypes (IgG, IgM, IgA) against Treponema pallidum (TP) (Standard Diagnostics Inc., South Korea) was used for screening for syphilis. In accordance with current practice, HIV testing was facility based and conducted as per national guidelines trhough a two-step algorithm using Determine™ HIV-1/2 (Abbott Laboratories, US) and Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech, UK).

3.10.1.3 Haemoglobin assessment

The cuvette tip was placed on to the end of a syringe needle that was used to draw blood from the specimen site and the blood allowed to be drawn into the cuvette by capillary action. Haemoglobin was then assessed using the HemoCue machine (HemoCue® B-Haemoglobin, Angelholm Sweden). On a daily basis, the calibration control cuvette specific for the HemoCue

photometer was tested before participants' testing. Values obtained were expected not to deviate from the assigned value on the control cuvette card by more than ±0.3g/dl.

Figure 3.2 First Response® Combo Malaria Ag (pLDH/HRP2) test procedures and interpretation of results



3.10.1.4 Urine based tests for proteinuria and glycosuria

As part of screening for preeclampsia and gestational diabetes, women had urine dipstick tests performed (Seimens Multistix 10SG, Seimens, UK). Urine dipsticks were also used as point of

care diagnostics for urinary tract infection and subsequent occurrences of hypertensive disorders in pregnancy.

3.10.2 Laboratory tests

3.10.2.1 Thick malaria blood smear

3.10.2.1.1 Preparation

A slide was first labelled with the participant's identification number, date of sample and the visit the sample was taken. 30 – 40 µl of whole blood was placed about 0.5 inches from the end of a 1 – by 3 – inch glass microscope slide on a horizontal surface. An applicator was then used to spread the blood down the glass slide in the opposite direction to form a blood film down the length of the slide. Films were allowed to air dry for between 30 minutes and 1 hour in the horizontal position and protected from dust. Slides were then stained with diluted Giemsa stain (1:50, vol/vol) for 50 min, rinsed in buffered water for 3 to 5 min and allowed to air dry.

3.10.2.1.2 Reporting and recording of results

All results were written in a laboratory source book and were recorded onto a separate results CRF for data entry. Once dry, a drop of immersion oil was applied to the center of the film using oil immersion objective (x 100 objective), an area that was stained and not too thick was selected and examined for the presence of asexual parasites. Examination of the slide consisted of counting against 200 high power fields before declaring the slide negative. Gametocytes were not counted as positive. If asexual stage parasites are identified before completing 200 high power fields, the number of parasites were then counted against the first 300 white blood cells, and the number of parasites against 300 white blood cells. Gametocytes were counted separately in a similar fashion.

A second microscopist ('Second Reader') read all the slides, blinded to the results of the first reader, and records their results in a Laboratory Book labeled 'second reader' and on the appropriate

laboratory form. In instances of discrepancies between the first microscopist ('First Reader') and the 'Second Reader', a third microscopist ('Third Reader') read the slides that had conflicting results: Level of discrepancy that justifies a third reader were: Positive vs negative, Species difference, >400% difference in count if 'First Reader' count is less than 1000/200 WBC and >200% difference in count if 'First Reader' count is more than or equal to 1000/WBC. The 'Third Reader' recorded their results in the Laboratory Book labeled 'third reader' and on the appropriate laboratory form. The result of the third reader will be used as definitive result. In house quality control measures were effected to ensure consistent quality of microscopy.

3.10.2.2 Placental impression smear microscopy

3.10.2.2.1 Preparation

At delivery, the placenta was cleaned with running water to clear the placenta of the remaining blood and mucus from its surface, and then place it onto a flat surface with the maternal side facing upwards. A full thickness section of the placental tissue located in an off-centre position half way between the insertion of the umbilical cord and the edge of the placenta, was cut out. A parallel incision 1 cm away from the first one was made in the placenta and a 1.5 cm3 section of placenta removed. The section was lightly dried with a small piece of filter paper (2.5x2.5 cm approximately) and carefully pressed along the surface of a glass microscope slide three times. The slide was labelled with the participant's identification number and date of the sample in pencil on the frosted area of the slide.

The smears were later fixed by dipping them in absolute methanol, which was followed by staining with diluted Giemsa stain (1:20, vol/vol) for 20 min. The slide was later washed briefly in buffered water and allowed to air dry before reading

3.10.2.2.2 Reporting and recording of results

The slide was examined under oil immersion objective with the number of parasitised red blood cells being counted against 500 red blood cells. Parasitaemia was reported as the proportion of parasitaemia as follows: Number of parasitised RBCs per 500 RBCs / 5 = % Parasitaemia.

3.10.2.3 Placental histology

3.10.2.3.1 Specimen preparation

Following initial cleaning of the placenta after delivery, a full thickness block of tissue about 2 cm x 2cm x 1 cm deep was cut out from a location in an off-centre position half way between the insertion of the umbilical cord and the edge of the placenta, free of infarcted areas, and place it into 30 mL 10% neutral buffered formalin in a 50 mL capped test tube at room temperature. Both the tube and lid were labeled with the participant identification number, the date and time of collection as well as date and time of delivery. Samples were not kept for more than 24 hours before being delivered to the histology laboratory for further processing and reading.

3.10.2.3.2 Further specimen preparation, reading and reporting of results

The placental sections were initially dehydrated through immersion in a series of alcohols, 70%, 90% and 100% for 2 hours in each concentration, followed by clearing in two serial baths of toluene for 2 hours each. The sections were then impregnated with paraffin wax through a series of 2 baths in liquid paraffin for 2 hours each. This was followed by embedding with molten wax and then sectioning with a rotary microtome. Sections were then placed on microscope slides and stained with haematoxylin and eosin.

Examination of placental histology slides and reporting were conducted as follows:

- The slides was first evaluated for the presence or absence of autolysis (characterised by poor preservation of the tissue, with absence of nuclei) and recorded as: 1=absent, 2=mild, 3=moderate, 4=severe.
- The presence or absence of formalin pigment was then examined (when present pigment is detected in a widespread location) and documented as: 1=absent, 2=mild, 3=moderate, 4=abundant
- The amount of maternal erythrocytes intervillous space was evaluated as : 1=absent, 2=scanty, 3=moderate, 4=abundant
- The presence or absence of deciduas basalis in the sample was evaluated as: 1=absent, 2=present
- The present or absence of amnion in the sample: 1=absent, 2=present
- The slide was then scanned at 400 magnification to examine for parasites in the intervillous space. If parasites were identified, the percentage of the parasitised erythrocytes was calculated and recorded.
- Hemozoin pigment was examined for at 100 magnification and subsequently repeated at magnification of 400 if none was identified. If pigment was identified, it's location was reported as either in
    - Free macrophages alone,
    - Fibrin alone, or
    - Both fibrin and free macrophages

  The amount of pigment was then quantified as either:
    - Mild: very few spots identified focally,
    - Moderate: small spots diffusely distributed or coarse deposits focally located, or
    - Abundant: large and coarse spots diffusely distributed

- Abnormalities in the intervillous space were evaluated. Intervillous inflammation was examined for by magnification of any areas with most white cells. Magnification of 400 was used to count the number of white cells per area:
    - 1=absent
    - 2=1-10%
    - 3=>10%

  Inflammatory cells in the villous stroma were examined and quantified as
    - 1=0-1
    - 2=2-5
    - 3=6-10
    - 4=>10
- The presence of calcifications was evaluated and reported as 1= absent, 2=minimal, 3=moderate, 4=abundant
- Chorioaminionitis was evaluated and reported as: 1=absent, 2=present, 3=not evaluable.

3.10.2.4 Polymerase chain reaction for P. falciparum detection

Dry blood spots for PCR assays were prepared with Whatman's #3 filter paper. The filter paper was labelled with the study ID number and date of sample. Using the pipette set at 40µl, approximately 40µl of whole blood was collected from a drawn blood sample and deposited in the center of the disc of Whatman's filter paper. This was repeated until the four spots had been filled. Once the blood spots were made, the filter paper was set onto clean paper and left on a shelf to air dry in a cabinet. Filter papers were never approximated to each other to ensure that there was no cross contamination among specimens.

Once dry, the filter papers were individually stored in separate re-sealable Ziploc plastic bag labelled with patients ID number and the contents kept dry with a single packet of desiccant added

to prevent sample degradation due to moisture. The filter-paper samples were stored at room temperature pending shipping.

Samples were tested in duplicate in a real-time PCR assay that targeted the *P. falciparum* lactate dehydrogenase gene (*pfldh*). This is a single-copy gene. Samples were run on a 384-well plate, and each run included 10 standards with 3d7 gDNA at concentrations from 0.1ng/µL to 5x10-6 ng/µL. Genomic DNA (gDNA) was extracted using a Chelex-100 protocol on three 5mm punches from a dried blood spot. gDNA specimens were tested in duplicate in a real-time PCR assay targeting the P. falciparum lactate dehydrogenase gene (pfldh) (242). Samples were tested on 384-well plates using a BioRad CFX384 Touch machine, threshold lines were set manually for each reaction plate, and quantitation cycle (Cq) values were computed using the Baseline subtracted curve fit setting. On each reaction plate was included a series of 10 standards in duplicate of P. falciparum strain 3D7 gDNA in concentrations from 0.1ng/uL to 5x10-6ng/uL; the Cq values of these controls were used to construct standard curves to estimate parasite gDNA quantity in the clinical specimens. Each plate also included four negative controls with molecular-grade water in place of DNA, and all plates were prepared in a PCR hood using filtered pipet tips.

3.11 **Data management**

Source documents were the participants' antenatal cards, delivery and birth records, laboratory results, and clinic records. All study-relevant data was transferred from the source documents to the case record forms (CRFs). In addition, some study specific data was recorded directly onto the CRF. Each participant had their own document file containing some of their original documents (e.g. print-out of automated laboratory results) and study specific CRFs. The investigators ensured that the CRFs were accurate, complete and legible.

The data recorded on the CRF was input into an electronic database using Cardiff Teleform Optical Mark Reader (OMR) v10.2 and managed by the data manager under the local PI in Malawi.

Data validation and verification were done to ensure that the data in the database corresponded with the CRFs and source documents. Data query sheets were raised and distributed by the data manager to the study team for resolution in a timely manner.

All documents were stored safely in confidential conditions. On all study-specific documents, other than the signed consent forms, the participant was referred to by their unique study participant number and initials, and not by name.

3.12 Safety monitoring and reporting of adverse events

Follow-up for symptomatic adverse events was focused on serious adverse events, adverse events serious enough to be reported to the study clinics through passive surveillance or requiring treatment, and the tolerability of the study drugs through direct observation at administration and as self-reported by the participants at visits.

3.12.1 Reporting procedures for all adverse events

An independent physician based in Malawi was appointed by the trial sponsor to act as an independent safety monitor. All adverse events occurring during the study, observed by the investigator or reported by the participant, whether or not attributed to study medication, were recorded using Adverse Event Forms. The following information was recorded: description, date of onset and end date, severity, seriousness, action taken and outcome of the event.

The relationship of adverse events to the study medication and the expectedness of each suspected adverse event according to the Summary of Product Characteristics for SP and the Investigators Brochure for DHA-PQ was assessed by a medically qualified investigator and the independent safety monitor. In the event that the ISM disagreed with the investigator's assessment, both opinions will be included in the report.

The investigator judged whether or not an adverse event was of sufficient severity to require discontinuation of the study treatment. If this occurred, or if the participant wished to withdraw due

to what they considered to be an intolerable adverse reaction, the participant was offered an end of study assessment and provided with appropriate medical care until symptoms ceased or the condition became stable.

3.12.2 Reporting procedures for serious adverse events

The investigator reported all serious adverse events (SAEs) that occur in the course of the study to the ISM, the Data Safety and Monitoring Board, the local pharmaceutical regulators (PMPB) and the ethics review board (NHSRC) within 24 hours of the investigational site becoming aware of the event. All SAEs will be reported, whether or not they were deemed to be causally related to the study drugs. All SAEs were reported using a standard SAE reporting form.

The immediate reports were followed within 24 hours by detailed, written reports with both reports identifying participants by their study ID number. In cases of doubt about whether an event fulfilled the criteria of serious, the case was reported to the ISM who proceeded to assess whether the event should be reported as an SAE.

All SAEs were reported by the investigator to the sponsor within 48 hrs of discovery or notification of the event. The sponsor was responsible for notifying the relevant external Ethics Committee (LSTM REC) and the trial Data Safety and Monitoring Board (DSMB). Detailed full SAE reports were provided to the DSMB to consider any action that may be needed in response to reported adverse events.

3.13 Overview of statistical methods for analysis

3.13.1 Efficacy of ISTp – DP vs. IPTp-SP for the control of malaria in pregnancy

The primary efficacy analysis was based on the assessment of the superiority of ISTp-DP over IPTp-SP in women in their first and second pregnancies for the prevention of the primary endpoint. Similar analyses were undertaken for the additional primary outcome for women in their third to fifth pregnancies, and for the secondary efficacy outcomes.

3.13.1.1 Analytical populations

The primary efficacy analysis was based on modified intention (mITT) to treat which included all women who had been randomized into the trial and for whom there was a study outcome of interest. Women who were randomized but later classified as screening failures were excluded. The second level of analysis was by per protocol (PP) in which only women who contributed to the endpoint of interest and either:

a. Received the study intervention (IPTp-SP or ISTp-DP) on at least three separate occasions at least four weeks apart and took >= 2/3 of the study doses on each occasion when measured; or
b. Reached a study end-point before completion of the three-visit schedule but received the intervention at least once; or
c. Received an approved alternative treatment for symptomatic malaria according to protocol that replaced the need for the scheduled intervention.

Women were excluded from the PP analysis if they did not adhere to the study treatments, used prohibited medications or were screening failures.

A safety population was defined in order to evaluate potential adverse events between intervention arms. This group constituted all women who had received the study intervention (i.e. either IPTp-SP or ISTp, with DP if ever diagnosed with malaria) with sufficient follow-up time, defined as attending the at least one subsequent scheduled study visit after receiving the first intervention dose at enrolment.

3.13.1.2 General analytical approach

Data from all three investigational sites was pooled as the study was conducted under a common protocol with the intention of pooling the data for analysis. All analysis was conducted and presented results stratified by gravidity group and overall (summary estimate for all gravidae

obtained by stratified analysis). Women were included in the gravidity strata that they were allocated to at enrolment.

Missing data for primary and secondary endpoints was not imputed. Missing values for 9 covariates selected a priori (likely prognostic: gravidity, participant enrolment site, malaria status at enrolment, season during pregnancy, maternal height; less likely prognostic: haemoglobin status at enrolment, socioeconomic status, corrected gestational age at enrolment and educational status) was imputed for the covariate adjusted analysis of the primary endpoint.

Generalised linear models were used where appropriate to assess the effect of the intervention according to different characteristics of the participants. Treatment effects were expressed in terms of relative risk and risk difference for dichotomous variables and mean difference for continuous variables, and the corresponding 95% confidence intervals. Superiority was defined as a positive treatment effect that is greater in the ISTp group than the IPTp group, where the 95% confidence interval does not include the point of no difference (RR value of 1).

Both modified intention-to-treat and per-protocol analyses were undertaken for the efficacy analyses. The modified intention to treat analysis was the primary analysis to assess superiority. In the absence of evidence for superiority, the prospectively defined non-inferiority margin was 15% for adverse outcome or placental malaria in the intention to treat and per protocol populations (relative difference between endpoints of 15% (i.e. RR 0.85); e.g. 24.5% versus 28.8% for adverse birth outcome) (333).

3.13.2 Effect of timing and frequency of submicroscopic parasitaemia and the reliability of RDTs for the detection of active placental malaria

Binomial regression model was used to evaluate the relationship between the frequency and timing of sub-diagnostic parasitaemia and low birth weight. Sensitivity/ specificity and positive/negative predictive values were used to assess the reliability of the pLDH/HRP2 rapid diagnostic test from the peripheral maternal blood and placental blood for

detecting active placental malaria against placental histology as the reference standard test. Further details on the statistical considerations and methods are provided in the respective chapters.

3.14 Ethical considerations

The Investigators ensured the study was conducted in full conformity with relevant regulations and with the International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP) (CPMP/ICH/135/95) July 1996. The protocol, informed consent forms and participant information sheets were submitted to the research ethics committees at the College of Medicine (Blantyre, Malawi), the National Health Science Research Committee of the Malawi, Ministry of Health, and the Liverpool School of Tropical Medicine for approval. Prior to initiating the trial, written and dated approval were obtained from all relevant ethics committees. Any protocol amendments were submitted to the ethics committees before implementation. Progress reports, SUSAR reports and safety reports were submitted in accordance with local requirements.

3.14.1 Informed consent and participant confidentiality

Informed consent was obtained before women were enrolled in the study as per Annex 2. The study staff ensured that participant anonymity was maintained. Participants were identified only by their study identification number and initials on the CRF and in any electronic database. All documents were stored securely and only accessible by study staff and other authorised personnel.

3.14.2 Other ethical considerations

3.14.2.1 Safety of the study drugs in pregnancy

Both SP and DHA-PQ are currently thought to be safe for the mother and foetus during the second and third trimesters of pregnancy. However, adverse events, particularly those associated with the study medication, were recorded and monitored throughout the trial.

3.14.2.2 Blood sampling

All examinations undertaken as part of this study were non-invasive, with the exception of blood sampling. Venous blood samples of 5 ml were taken as described in the trial procedures. Blood sampling was likely inconvenient to the participants, and caused minor discomfort and bruising. In some aspects of the trial, venous blood sampling had the potential to directly benefit the participants or their babies as any malaria infection or anaemia detected as a result of the sampling would be treated as well as the avoiding of repeated finger pricking, which was deemed more uncomfortable.

The overall volume of blood collected from each participant was small, a maximum of 20 ml per woman over the course of the study. Only well trained nursing and laboratory staff were employed on the trial and new disposable needles and lancets will used for blood taking procedures, being safely discarded immediately after their use.

3.14.2.3 Inclusion of young people under the age of 18

This study included young women below the age of 18 years. In Malawi, young pregnant women aged 15 or older are considered emancipated, and are legally able to consent on their own behalf to be included in a clinical trial. It was important to include young women in the trial, as adolescents are known to be particularly susceptible to malaria in pregnancy, and are therefore one of the groups that may potentially benefit the most from any improvements to practice in preventing adverse outcomes related to malaria in pregnancy.

3.14.2.4 Reimbursement of costs

The study provided all study drugs, study procedures, study-related visits and reasonable medical expenses that were incurred as a result of the study including expenses for transport and a meal allowance for any study visits which were conducted over lunch times.

3.15 Trial monitoring and GCP compliance (Quality assurance procedures)

The study was conducted in accordance with the current approved protocol, ICH GCP, relevant regulations and standard operating procedures. Regular monitoring was performed in accordance with ICH GCP. The Research Support Centre of the College of Medicine provided the clinical monitoring on behalf of the sponsor with monitoring visits conducted prior to enrolment, shortly after the start of enrolment, and at periodic intervals during the trial, to ensure conduct according to the published protocol and any amendments.

4 CHAPTER FOUR: SCHEDULED INTERMITTENT SCREENING AND TREATMENT IN PREGNANCY (ISTP) FOR THE CONTROL OF MALARIA IN PREGNANCY IN MALAWI: A RANDOMIZED CONTROLLED TRIAL

4.1 Results

4.1.1 Baseline and patient disposition

Between 21 July 2011 and 18 March 2013, 3,214 women were screened for inclusion; 1,873 women were randomized (paucigravidae=1155; multigravidae=718) and 1,743 (93.1%) were seen at delivery (Figure 4.1). Overall 6,504 of 6,942 (93.7%) scheduled antenatal follow-up visits were attended (Table 4.1) and 1,742 women (94.5%) attended all scheduled visits. Ultimately, 1,702 (90.9% (ISTp=90.6%, IPTp=91.2%) contributed to the primary morbidity outcome and 1,653 (88.3%) (ISTp=88.6%, IPTp=87.9%) to the primary outcome of malaria infection at delivery. The baseline characteristics were well balanced, within each gravidity strata and overall (Table 4.2). At baseline, about half of the women were infected with malaria parasites. In both arms, the median (IQR) follow-up time was 4.0 (3.2-4.7) months and median (range) number of scheduled visits was 4 (1-4) (Table 4.1). In the ISTp-DP arm, 48.8%, 38.0%, 12.4% and 0.9% received 0, 1, 2, 3, courses of DP, respectively (Table 4.1).

4.1.2 Primary outcome

Among paucigravidae, the prevalence of SGA/LBW/PT was similar in the ISTp-DP (33.7%) and IPTp-SP (30.6%) arms (RR=1.10, 95% CI 0.92-1.31, p=0.282, Figure 4.2). The prevalence was also similar between arms among multigravidae.

Among multigravidae, the risk of malaria at delivery was significantly higher in the ISTp-DP (34.9%) than in the IPTp-SP (27.2%) arm (RR=1.29 (1.02-1.63), p=0.037). This was also evident among paucigravidae and all gravidae. In absolute terms, the risk of malaria was increased in multigravidae by 7.8% (0.6-14.9) and amongst all gravidae by 7.9% (3.1-12.6) (Figure 4.2).

Similar results for both primary outcomes were obtained from covariate adjusted analyses, with and without imputation for missing values (Table 4.3), with per protocol population analysis (Table 4.4) and following sensitivity analysis that restricted analysis to birthweight obtained within

24 hours of delivery (Table 4.5). Results for the primary composite live birth outcome were also consistent across subgroups, including study sites, (Figures 4.3 and 4.4), although the increased risk of malaria at delivery appeared lowest in primigravidae (Figure 4.4). However, Chikwawa and Madziabango, both high transmission areas, had significantly higher risk of primary outcome malaria infection at delivery, with no significant association noted for Mpemba (Figure 4.4).

4.1.3 Secondary efficacy outcomes

Following enrolment, 45.8% had >=1 episode of malaria infection prior to delivery (PCR, microscopy or RDT) and 11.4% had >=1 episode of clinical malaria. These were similar in both arms (Figure 4.5**Error! Reference source not found.**). At delivery, 22.2% of women had peripheral alaria detected by PCR, RDT or microscopy. This was higher in the ISTp-DP arm (RR=1.34 [1.12-1.61], p=0.002, Figure 4.5), particularly for sub-patent infections (PCR-positive, RDT or microscopy-negative, Figure 4.6). The overall prevalence of placental malaria detected by histology, PCR, RDT or microscopy was 38.0% and higher in the ISTp-DP arm: RR=1.16 (1.03-1.32), p=0.018 (Figure 4.5). The greatest difference was observed for acute rather than chronic or past histological infections (Figure 4.7). Congenital malaria was common (12.0%) in both groups (Figure 4.8).

At delivery, the mean haemoglobin concentration was higher (Table 4.6), and the prevalence of anaemia (Hb<11g/dL) lower in paucigravidae in the ISTp-DP arm relative to the ITPp-SP arm (Figure 4.5). The individual components of the primary endpoint are provided in Figure 4.8. LBW was more common in the ISTp-DP arm (RR=1.29 [0.97-1.71], p=0.079).

4.1.3.1 Exploratory analysis of the effect of last antimalarial dosing prior to delivery on the risk of active malaria infection at delivery

There was no significant difference to the time of delivery from dosing of an antimalarial at the last visit prior to delivery between the SP, AL or DP (Table 4.7). Women who received DP and AL at the last visit prior to delivery had a higher risk of peripheral malaria compared to women who

received SP (RR=1.74 [1.16, 2.59], p=0.007 and RR=3.20 [2.09, 4.90], p<0.001 respectively, Table 4.8). Receiving DP at the last visit was associated with a 46% lower risk of peripheral malaria at delivery than AL (29.9% vs 55.0%; RR=0.54 [0.32, 0.93], p=0.027 (Table 4.8).

Though not significant, there was an increased risk of active placental malaria in women who took DP than those who took SP at the last visit prior to delivery (RR=1.32 [0.99, 1.77], p=0.061, Table 4.8) whilst AL was significantly associated with a higher risk of active placental malaria when compared to SP (RR=2.25 [1.72, 2.96], p<0.001). Receipt of DP was significantly associated with a lower risk of active placental malaria at delivery when compared to AL (RR=0.59 [0.40, 0.85], p=0.005).

4.1.3.2 Risk of reinfection at the next scheduled clinic visit after treatment with dihydroartemisinin-piperaquine

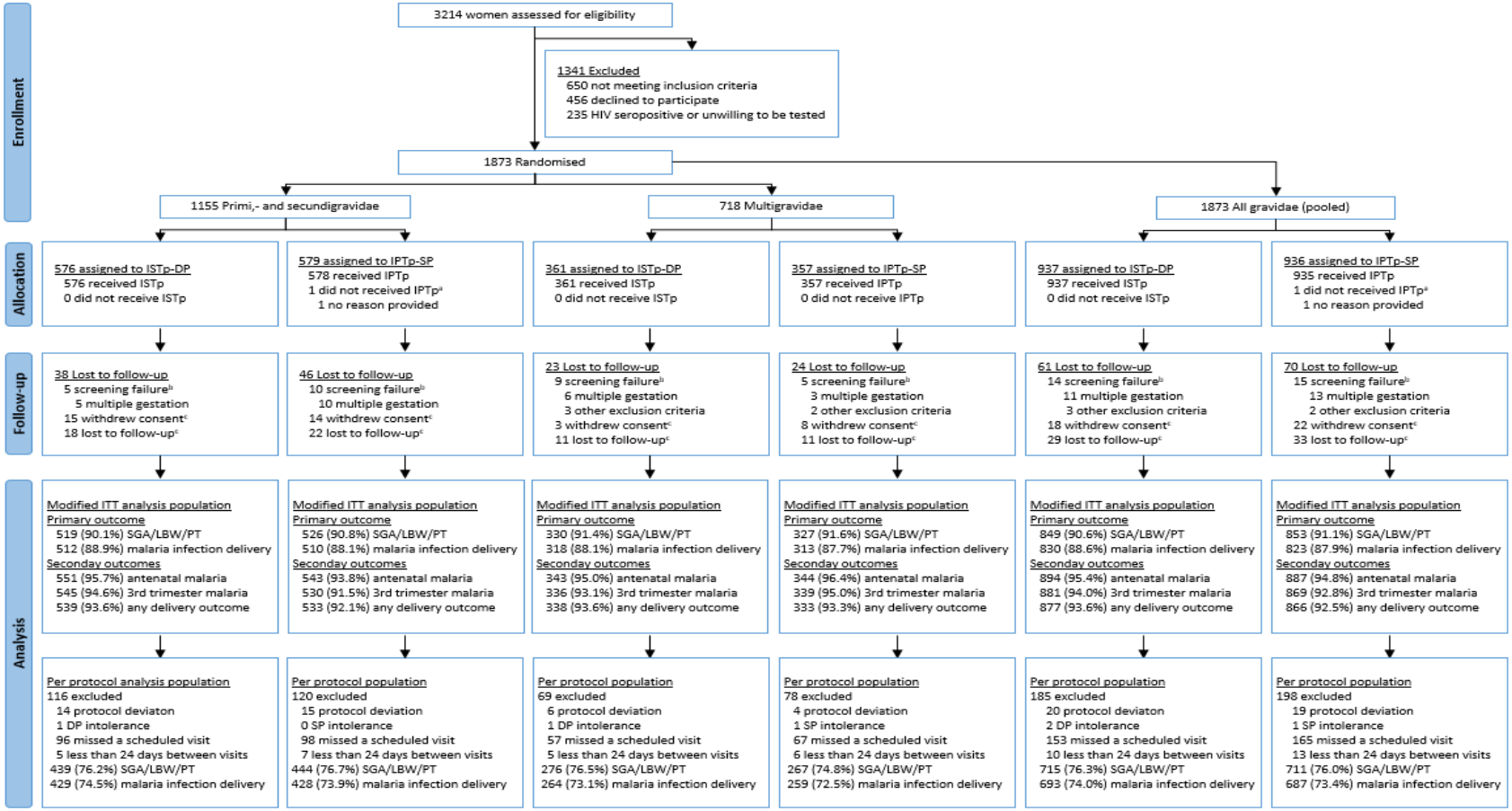
Overall, 17% of women ever treated with dihydroartemesinin-piperaquine at a scheduled antenatal visit presented with reinfection by either malaria microscopy, RDT or PCR by the time of their next antenatal visit (Table 4.9). Though the risk of reinfection progressively increased, the absolute number of women being treated at each visit decreased.

4.1.4 Adherence, tolerance, foetal loss, mortality and other safety outcomes

Overall DP was well tolerated (Table 4.10). There was no difference between arms in the number of maternal SAEs or deaths (Table 4.11). There were no severe cutaneous reactions. Foetal loss was highest in the ISTp-DP arm (2.6% vs 1.3%, Figure 4.8). Further stratified analysis within the ISTp-DP arm showed this was highest among women who had never received DP (i.e. pregnancies who remained RDT-negative throughout) (3.1% vs 2.2% in DP recipients, Table 4.11). Perinatal, neonatal and infant (by 6-8 weeks) mortality were also higher in the ISTp-DP arm (not significant) (Figure 4.8). Overall, foetal loss or infant death by the end of the follow-up period at 6-8 weeks occurred more often in the ISTp-DP (4.0%) than in the IPTp-SP (2.3%) arm (RR=1.76 [1.04-2.98],

p=0.036, Figure 4.4, Table 4.11). One case of neonatal jaundice was detected in the ISTp-DP arm (non DP-recipient) and none in the IPTp-SP arm. The frequency of congenital malformations was 1.5% and 1.0% in the ISTp-DP and ISTp-SP arm respectively (RR=1.44 (0.62-3.35), p=0.398) (1.5% vs 1.6% in non-DP vs DP-recipients within the ISTp-DP arm).

Figure 4.1:Participant flowchart



ITT=Intention to treat, SGA/LBW/PT=composite of small-for-gestational Age (SGA), or low birthweight (LBW), or preterm (PT).

a. One woman randomized to IPTp-SP was erroneously recorded as being in the ISTp-arm on her ANC card and as a result received ISTp-DP. She was included in the ITT population under the IPTp-SP arm.
b. Screening failures were not followed to delivery and excluded from the modified ITT population
c. Women lost to follow-up prior to delivery and women who withdrew consent were included in the modified ITT population and contributed to the antenatal follow-up analyses (e.g. incidence of malaria).

Table 4.1 Follow-up visits schedule (modified intention to treat population)

	Paucigravidae		Multigravidae		All gravidae	
	ISTp-DP (n=571)	**IPTp-SP (n=569)**	**ISTp-DP (n=352)**	**IPTp-SP (n=352)**	**ISTp-DP (n=923)**	**IPTp-SP (n=921)**
Planned No. of scheduled visits, including enrolment, excluding delivery,[a] No. (%)						
3	58 (10.2)	58 (10.2)	50 (14.2)	48 (13.6)	108 (11.7)	106 (11.5)
4	513 (89.8)	511 (89.8)	302 (85.8)	304 (86.4)	815 (88.3)	815 (88.5)
Total visits	2226	2218	1358	1360	3584	3578
Possible No. of scheduled visits adjusted for early delivery, including enrolment, excluding delivery,[b] No. (%)						
1	4 (0.4)	5 (0.9)	0 (0)	0 (0)	4 (0.4)	5 (0.9)
2	19 (3.3)	15 (2.6)	11 (3.1)	8 (2.3)	30 (3.3)	23 (2.5)
3	90 (15.8)	79 (13.9)	64 (18.2)	68 (19.3)	154 (16.7)	147 (16.0)
4	458 (80.2)	470 (82.6)	277 (78.7)	276 (78.4)	735 (79.6)	746 (81.0)
Total visits	2144	2152	1322	1324	3466	3476
Achieved number of scheduled visits, including enrolment, excluding delivery, No. (%)						
1	21 (3.7)	27 (4.7)	12 (3.4)	8 (2.3)	33 (3.6)	35 (3.8)
2	38 (6.7)	34 (6.0)	22 (6.3)	26 (7.4)	60 (6.5)	60 (6.5)
3	133 (23.3)	115 (20.2)	80 (22.7)	100 (28.4)	213 (23.1)	215 (23.3)
4	379 (66.4)	393 (69.1)	238 (67.6)	218 (61.9)	617 (66.8)	611 (66.3)
Total visits	2012	2012	1248	1232	3260	3244
Number of DP or SP courses received, No. (%)						

0	rev	1 (0.2)	230 (65.3)	0 (0)	450 (48.8)	1 (0.1)
1	244 (42.7)	29 (5.1)	107 (30.4)	11 (3.1)	351 (38.0)	40 (4.3)
2	99 (17.3)	39 (6.9)	15 (4.3)	27 (7.7)	114 (12.4)	66 (7.2)
3	8 (1.4)	119 (20.9)	0 (0.0)	103 (29.3)	8 (0.9)	222 (24.1)
4	0	381 (67.0)	0	211 (59.9)	0	592 (64.3)
Total courses received	466	1988	137	1218	603	3206
Person days contributed till delivery or till lost to follow-up, median (IQR)						
	117 (97-143)	117 (102-142)	115 (98-139)	116 (95-141)	116 (97-142)	117 (99-142)

The number of scheduled visits was dependent on the gestational age at enrolment. This was either 4 visits (including enrolment) if women were enrolled between 16 to 25 weeks gestation, or 3 visits if they were enrolled between 26 and 28 weeks gestation.

Adjusted for early delivery (i.e. excludes all planned antenatal visits that could not have occurred because the pregnancy ended before that scheduled date)

IQR=interquartile range

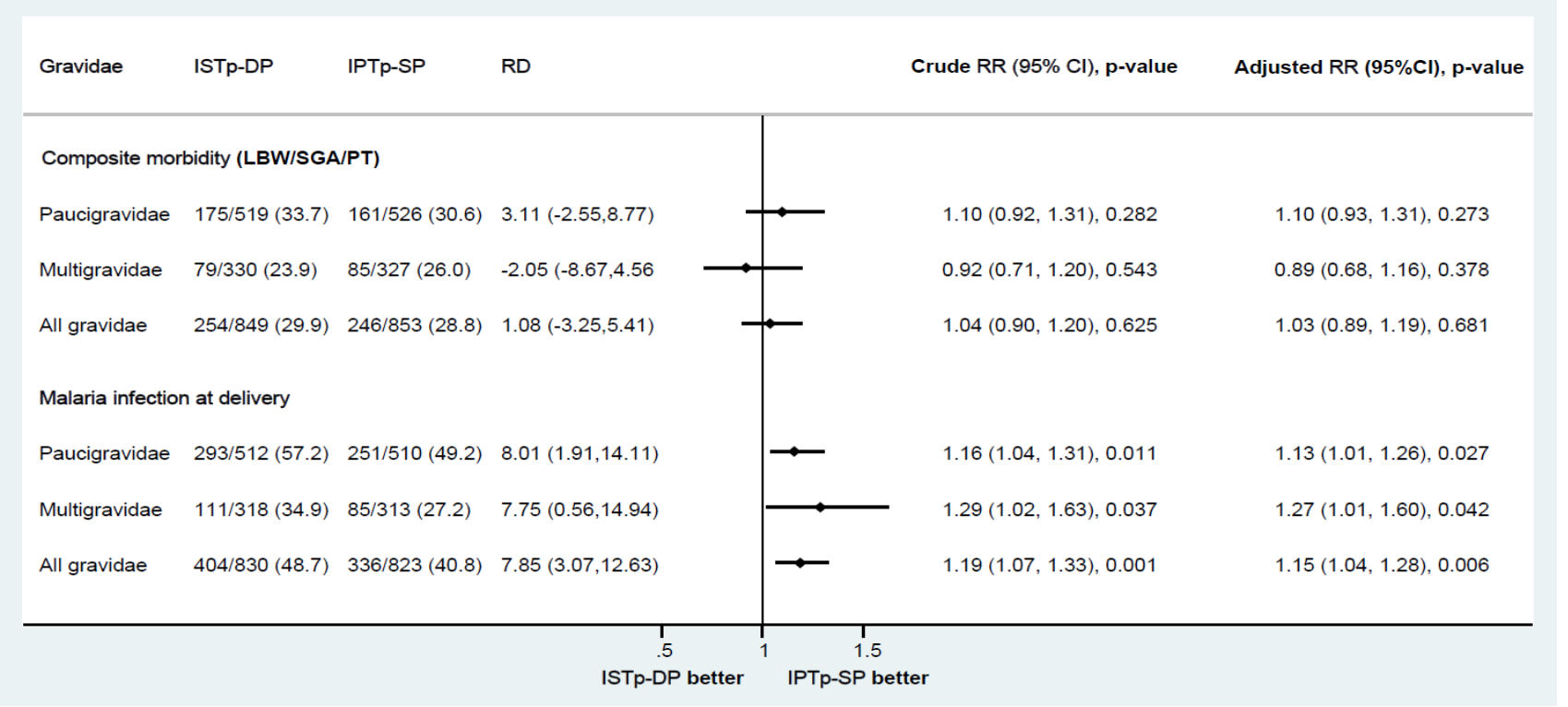
Table 4.2 Baseline characteristics (modified intention to treat population)

	Paucigravidae		Multigravidae		All gravidae (pooled)	
	ISTp-DP (n=571)	**IPTp-SP (n=569)**	**ISTp-DP (n=352)**	**IPTp-SP (n=352)**	**ISTp-DP (n=923)**	**IPTp-SP (n=921)**
Maternal characteristics						
Study site						
Madziabango	21.9% (125/571)	22.1% (126/569)	31.3% (110/352)	30.4% (107/352)	25.5% (235/923)	25.3% (233/921)
Mpemba	51.5% (294/571)	51.5% (293/569)	54.3% (191/352)	55.4% (195/352)	52.6% (485/923)	53.0% (488/921)
Chikwawa	26.6% (152/571)	26.4% (150/569)	14.5% (51/352)	14.2% (50/352)	22.0% (203/923)	21.7% (200/921)
Maternal age (years)	19.5 (2.7)	19.6 (2.8)	27.3 (4.3)	27.5 (4.2)	22.5 (5.1)	22.6 (5.1)
Marital status						
Single	8.1% (46/570)	8.8% (50/569)	2.0% (7/351)	0.3% (1/352)	5.8% (53/921)	5.5% (51/921)
Married	91.9% (524/570)	91.2% (519/569)	97.7% (343/351)	99.1.% (349/352)	94.1% (867/921)	94.2% (868/921)
Widowed/separated/divorced	0.0% (0/570)	0.0% (0/569)	0.3% (1/351)	0.6% (2/352)	0.1% (1/921)	0.2% (2/921)
Used a bednet last night	18.2% (104/571)	17.0% (97/569)	18.2% (64/351)	24.1% (85/352)	18.2% (168/922)	19.8% (182/921)
Schooling (years completed)	6.7 (3.4)	6.7 (3.3)	4.4(3.4)	4.4(3.9)	5.8(3.6)	5.9(3.7)
SES index score (terciles)						
Low	34.7% (198/570)	33.3% (189/567)	33.3% (117/351)	31.5% (111/352)	34.2% (315/921)	32.6% (300/919)
Medium	31.2% (178/570)	32.1% (182/567)	37.0% (130/351)	36.1% (127/352)	33.4% (308/921)	33.6% (309/919)
High	34.0% (194/570)	34.6% (196/567)	29.6% (104/351)	32.4% (114/352)	32.4% (298/921)	33.7% (310/919)

Rainfall 3 months before enrolment (average/month in millimetre)	67.2 (86.5)	66.1 (86.0)	67.6 (79.8)	66.6 (79.2)	67.3 (84.0)	66.3 (83.5)
Pregnancy number (gravidity)						
First	54.5% (311/571)	55.5% (316/569)	NA	NA	33.8% (311/921)	34.3% (316/920)
Second	45.5% (260/571)	44.5% (253/569)	NA	NA	28.2% (260/921)	27.5% (253/920)
Third	NA	NA	44.0% (154/352)	43.0% (151/351)	16.7% (154/921)	16.4% (151/920)
Fourth or higher	NA	NA	56.0% (196/352)	57.0% (200/351)	21.3% (196/921)	21.7% (200/920)
Gestational age by ultrasound (days)	145.1(23.0)	144.5(22.4)	149.7(23.7)	149.1(23.8)	146.8(23.3)	146.2(23.0)
Had a previous stillbirth/abortions	5.1% (29/571)	4.0% (23/569)	14.2% (50/352)	10.8% (38/352)	8.6% (79/923)	6.6% (61/921)
Maternal weight (kg)	54.1 (6.8)	54.4 (6.9)	56.1 (7.5)	57.0 (8.5)	54.8 (7.2)	55.4 (7.6)
Maternal height (cm)	154 (4.8)	154 (5.0)	154 (5.2)	155 (5.0)	154 (5.0)	154 (5.0)
Laboratory findings						
Haemoglobin (g/dL)	10.7 (1.5)	10.7 (1.5)	11.5 (1.3)	11.5 (1.3)	11.0 (1.5)	11.0 (1.4)
Malaria infection						
RDT	43.8% (250/571)	NA	19.4% (68/351)	NA	34.5% (318/922)	NA
Microscopy	17.7% (100/565)	20.8% (117/562)	11.8% (41/347)	9.4% (33/351)	15.5% (141/912)	16.4% (150/913)
PCR	51.3% (285/556)	51.7% (290/561)	33.1% (115/347)	28.7% (98/342)	44.3% (400/903)	43.0% (388/903)
Microscopy or PCR	52.5% (300/571)	54.9% (312/568)	36.4% (128/352)	31.5% (111/352)	46.4% (428/923)	46.0% (423/920)
Parasite density[a] (median, IQR)	1,000 (320-7,200)	2,427 (507-9,600)	320 (160-3,200)	907 (213-4,267)	800 (267-5,867)	2,400 (320-8,000)

Data are % (n/N), median (IQR), or mean (SD), unless otherwise indicated.
SES=Socioeconomic Status, mm=millimetres rainfall (average per month), kg=kilograms, cm=centimetre, g/dL:=grams per decilitre, RDT=Rapid diagnostic test for malaria, PCR=Polymerase Chain Reaction, NA=Not Applicable
Parasite density per/microliter assessed by microscopy

Figure 4.2Efficacy of ISTp-DP versus IPTp-SP on the composite primary outcomes of SGA, LBW and preterm birth and of malaria infection at delivery



RR=Relative Risk, RD=crude Risk Difference. Adjusted RR obtained from multivariate log binomial regression models with missing values imputed and adjusting for study site, and 7 pre-specified covariates: malaria status at enrolment (binary), season during pregnancy (terciles based on average ranked rainfall during the last 6 month of pregnancy), maternal height (terciles), haemoglobin status at enrolment (terciles), maternal years of schooling (terciles), socio-economic status (terciles of SES Index calculated using Principal Component Analysis (PCA), gestational age at booking (binary based on median).

Table 4.3 Modified intention to treat analysis population, co-variate adjusted analysis of primary endpoint, with and without imputation for missing variables

	no/No (%) of patients with events		Co-variate adjusted analysis No imputation for missing values		Co-variate adjusted analysis Missing values for co-variates imputed	
Outcome	**ISTp-DP**	**IPTp-SP**	**Risk Ratio (95% CI), p-value**	**Risk Difference (95% CI), p-value**	**Risk Ratio(95% CI), p-value**	**Risk Difference (95% CI), p-value**
SGA/LBW/PT						
Paucigravidae	175/519 (33.7)	161/526 (30.6)	1.10 (0.93, 1.31), 0.2727	2.17 (-3.34, 7.69), 0.4399	1.10 (0.93, 1.31), 0.2552	2.27 (-3.23, 7.77), 0.4189
Multigravidae	79/330 (23.9)	85/327 (26.0)	0.89 (0.68, 1.16), 0.3778	-3.96 (-10.55, 2.63), 0.2391	0.89 (0.68, 1.16), 0.3778	-3.96 (-10.55, 2.63), 0.2391
All gravidae	254/849 (29.9)	246/853 (28.8)	1.03 (0.89, 1.19), 0.6814	0.04 (-4.23, 4.32), 0.9856	1.03 (0.89, 1.19), 0.6594	0.10 (-4.17, 4.37), 0.9638
Malaria infection at delivery						
Paucigravidae	293/512 (57.2)	251/510 (49.2)	1.13 (1.01, 1.26), 0.0266	7.88 (1.97, 13.80), 0.0090	1.13 (1.02, 1.27), 0.0237	8.03 (2.13, 13.94), 0.0077
Multigravidae	111/318 (34.9)	85/313 (27.2)	1.27 (1.01, 1.60), 0.0419	8.17 (1.02, 15.32), 0.0252	1.27 (1.01, 1.60), 0.0419	8.17 (1.02, 15.32), 0.0252
All gravidae	404/830 (48.7)	336/823 (40.8)	1.15 (1.04, 1.28), 0.0060	7.21 (2.59, 11.84), 0.0022	1.15 (1.04, 1.28), 0.0055	7.27 (2.65, 11.89), 0.0021

Table 4.4 Per protocol analysis population, unadjusted and co-variate adjusted analysis of primary endpoint, with missing values for co-variates imputed

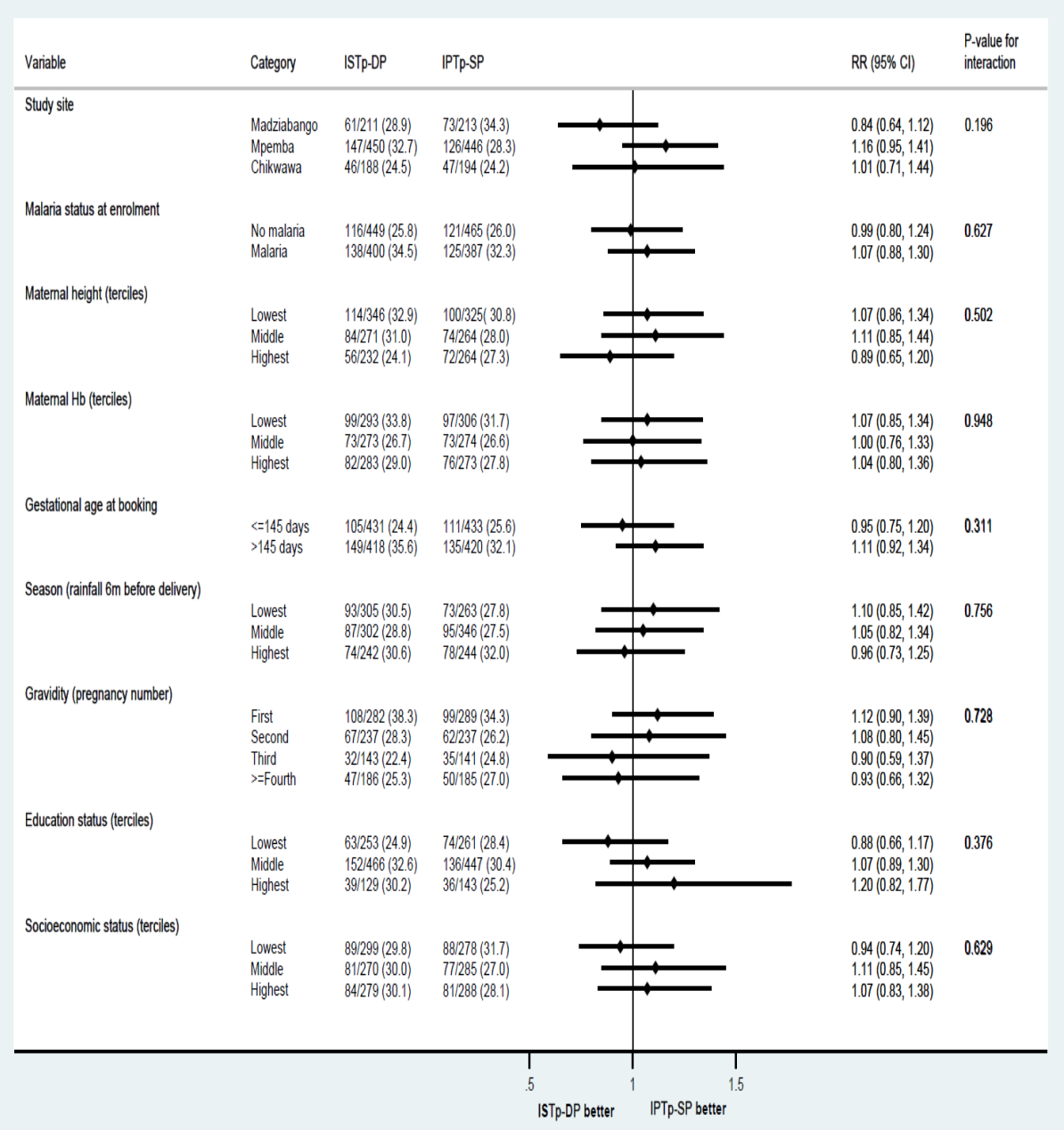
	no/No (%) of patients with events		Unadjusted analysis		Co-variate adjusted analysis	
Outcome	**ISTp-DP**	**IPTp-SP**	**Risk Ratio (95% CI), p-value**	**Risk Difference (95% CI), p-value**	**Risk Ratio (95% CI), p-value**	**Risk Difference (95% CI), p-value**
SGA/LBW/PT						
Paucigravidae	123/439 (28.0)	109/444 (24.5)	1.16 (0.93, 1.43), 0.1869	3.05 (-2.57, 8.67), 0.2874	1.16 (0.94, 1.44), 0.1738	3.16 (-2.43, 8.76), 0.2679
Multigravidae	55/276 (19.9)	55/267 (20.6)	0.94 (0.68, 1.32), 0.7326	-2.60 (-9.51, 4.32), 0.4619	0.94 (0.68, 1.32), 0.7326	-2.60 (-9.51, 4.32), 0.4619
All gravidae	178/715 (24.9)	164/711 (23.1)	1.08 (0.90, 1.29), 0.4253	1.18 (-3.14, 5.51), 0.5917	1.08 (0.90, 1.29), 0.4101	1.22 (-3.10, 5.55), 0.5789
Malaria infection at delivery						
Paucigravidae	241/429 (56.2)	199/428 (46.5)	1.21 (1.06, 1.38), 0.0048	9.83 (3.42, 16.25), 0.0027	1.19 (1.05, 1.35), 0.0059	10.00 (3.60, 16.40, 0.0022
Multigravidae	88/264 (33.3)	64/259 (24.7)	1.35 (1.03, 1.77), 0.0314	8.58 (0.76, 16.40), 0.0315	1.36 (1.03, 1.78), 0.0273	8.58 (0.76, 16.40), 0.0315
All gravidae	329/693 (47.5)	263/687 (38.3)	1.24 (1.10, 1.40), 0.0006	8.70 (3.69, 13.72), 0.0007	1.20 (1.07, 1.35), 0.0017	8.77 (3.76, 13.78), 0.0006

Table 4.5 Sensitivity analysis to determine the effect of the use of corrected versus uncorrected birthweight, using the intention to treat analysis population

	Corrected birthweight within 7 days of birth			Un-corrected birthweight measured within 24 hours of delivery		
	no/No (%) of women with events or No, mean (SD)		Risk Ratio or Mean Difference	no/No (%) of women with events or No, mean (SD)		Risk Ratio or Mean Difference
Outcome	**ISTp-DP**	**IPTp-SP**	**(95% CI), p-value**	**ISTp-DP**	**IPTp-SP**	**(95% CI), p-value**
Birthweight						
Paucigravidae	504; 2,859 (412)	508; 2,891 (454)	-32.32 (-85.72, 21.08), 0.2355	480; 2,853 (406)	488; 2,887 (453)	-34.81 (-88.93, 19.30), 0.2073
Multigravidae	314; 3,020 (421)	310; 3,041 (404)	-20.56 (-85.23, 44.11), 0.5333	293; 3,010 (418)	298; 3,035 (403)	-25.03 (-91.13, 41.08), 0.4581
All gravidae	818; 2,921 (423)	818; 2,948 (442)	-27.07 (-68.95, 14.80), 0.2051	773; 2,912 (417)	786; 2,944 (440)	-31.12 (-73.68, 11.44), 0.1518
LBW						
Paucigravidae	77/504 (15.3)	59/508 (11.6)	1.32 (0.96, 1.80), 0.0890	74/480 (15.4)	56/488 (11.5)	1.34 (0.97, 1.86), 0.0736
Multigravidae	21/314 (6.7)	17/310 (5.5)	1.22 (0.66, 2.27), 0.5303	19/293 (6.5)	16/298 (5.4)	1.21 (0.63, 2.30), 0.5663
All gravidae	98/818 (12.0)	76/818 (9.3)	1.29 (0.97, 1.71), 0.0788	93/773 (12.0)	72/786 (9.2)	1.31 (0.98, 1.76), 0.0665
SGA/LBW/PT						
Paucigravidae	175/519 (33.7)	161/526 (30.6)	1.10 (0.92, 1.31), 0.2822	175/519 (33.7)	160/526 (30.4)	1.11 (0.93, 1.32), 0.2535
Multigravidae	79/330 (23.9)	85/327 (26.0)	0.92 (0.71, 1.20), 0.5431	79/330 (23.9)	85/327 (26.0)	0.92 (0.71, 1.20), 0.5431
All gravidae	254/849 (29.9)	246/853 (28.8)	1.04 (0.90, 1.20), 0.6254	254/849 (29.9)	245/853 (28.7)	1.04 (0.90, 1.21), 0.5881

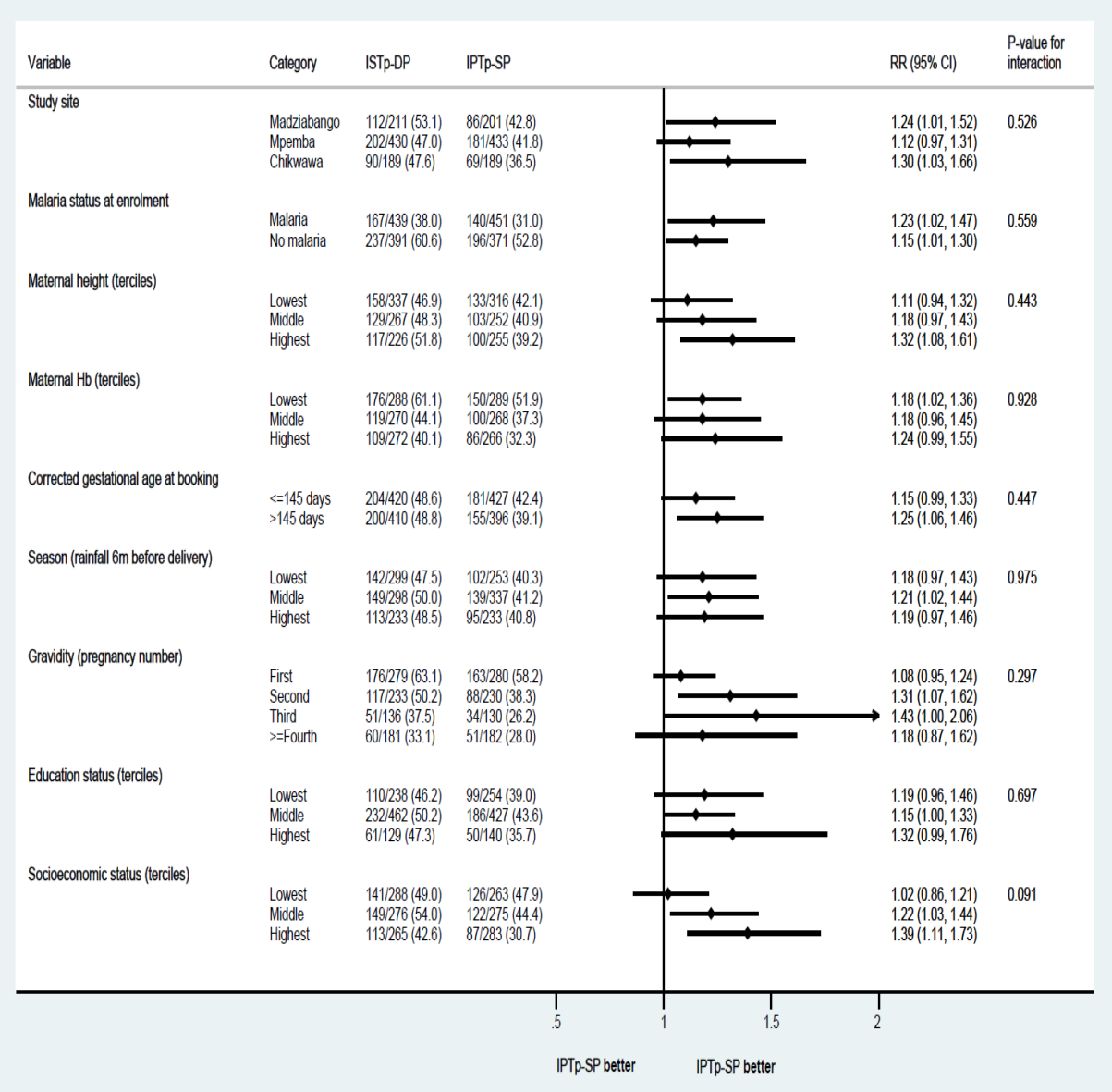
All birthweight in the primary analyses refer to corrected birthweights taken within 7 days (168 hours) after birth. Birthweights taken more than 24 hours after delivery were corrected for the physiological fall in birth weight in breastfed infants occurring in the first days following deliver (334, 335). Birth weights taken 24-48h hours, and 48-168 hours after delivery were corrected by a factor +2% and +4%, respectively to obtain the estimated weight at birth (336, 337) .

Figure 4.3Subgroup analysis of the effect of ISTp-DP versus IPTp-SP on the composite primary outcomes of SGA, LBW and preterm birth



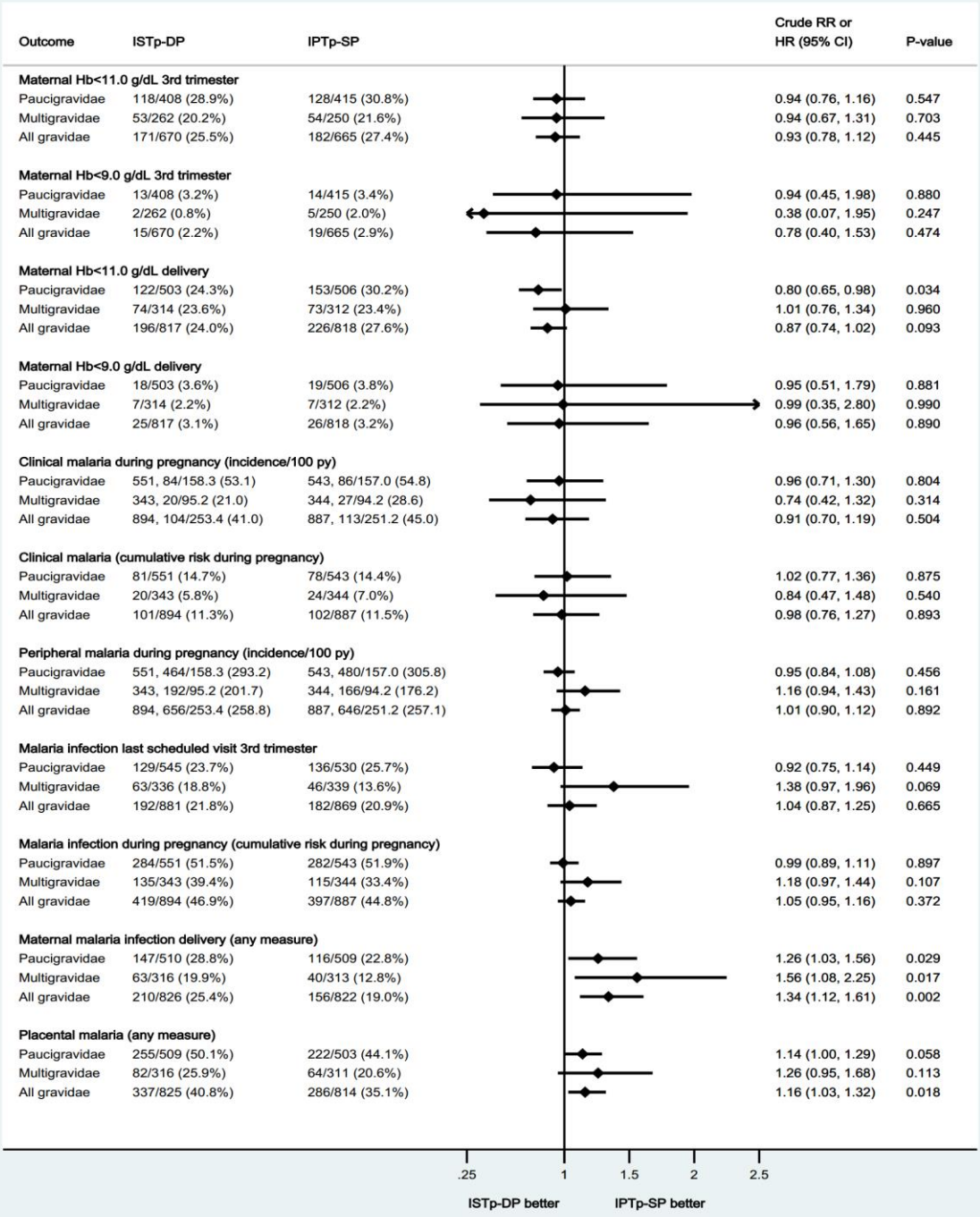
RR=Relative Risk, Hb=haemoglobin, Season was defined by ranking the average rainfall in the 6 months prior to delivery.

Figure 4.4 Subgroup analysis of the effect of ISTp-DP versus IPTp-SP on the primary outcome malaria infection at delivery



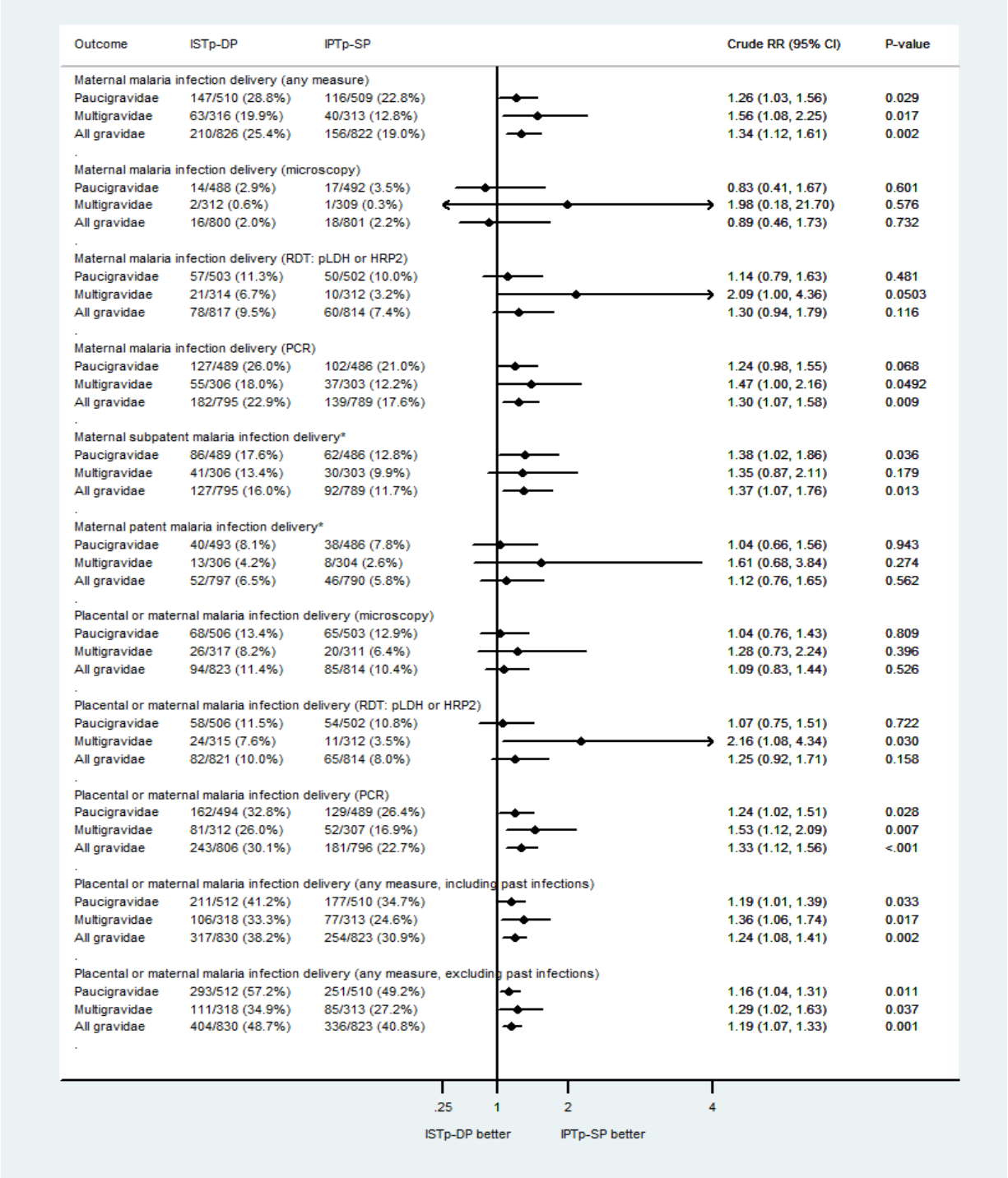
RR=Relative Risk, Hb=haemoglobin, Season was defined by ranking the average rainfall in the 6 months prior to delivery. The difference in treatment effect for malaria infection at delivery was greater in multigravidae and secundigravidae pooled (RR=1.30) than in primigravidae (RR=1.08) (p=0.08 for difference between subgroups).

Figure 4.5 Maternal secondary outcomes: anaemia and malaria



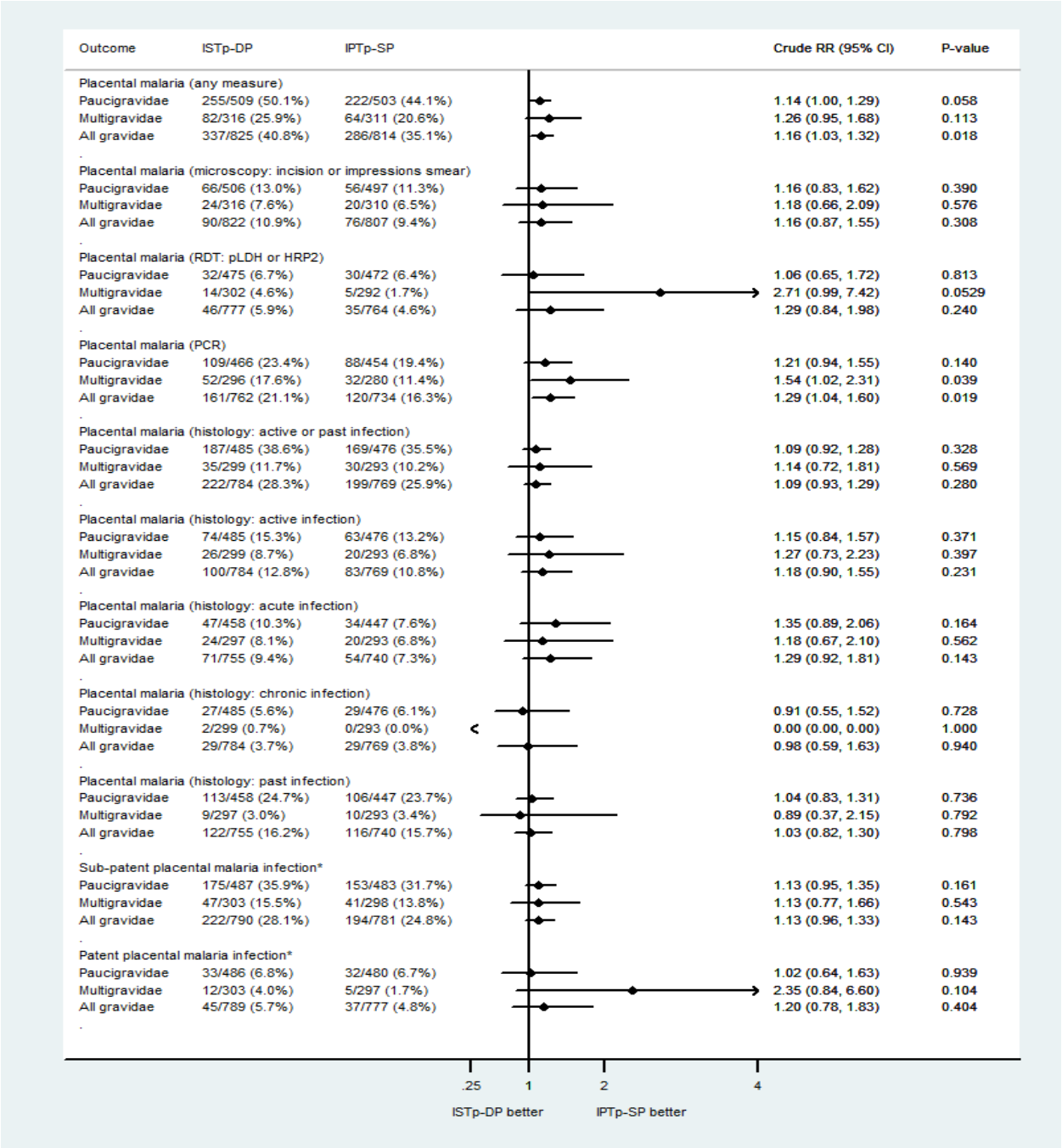
a. Data for the incidence of malaria infection and clinical malaria represent the number of women with an event, the number of events, the person time follow-up and in brackets, the incidence rate per 100 person years

Figure 4.6 Patent and sub-patent malaria infection at delivery by microscopy, RDT or PCR



* PCR positive and RDT or microscopy positive (patent) or RDT or microscopy negative (sub-patent)

Figure 4.7 Placental malaria infection by microscopy, RDT, PCR and placental histology



* PCR positive or histology positive (active infection) and RDT or microscopy positive (patent) or RDT or microscopy negative (sub-patent)

Figure 4.8 Secondary outcomes newborn: birth outcomes and neonatal follow-up

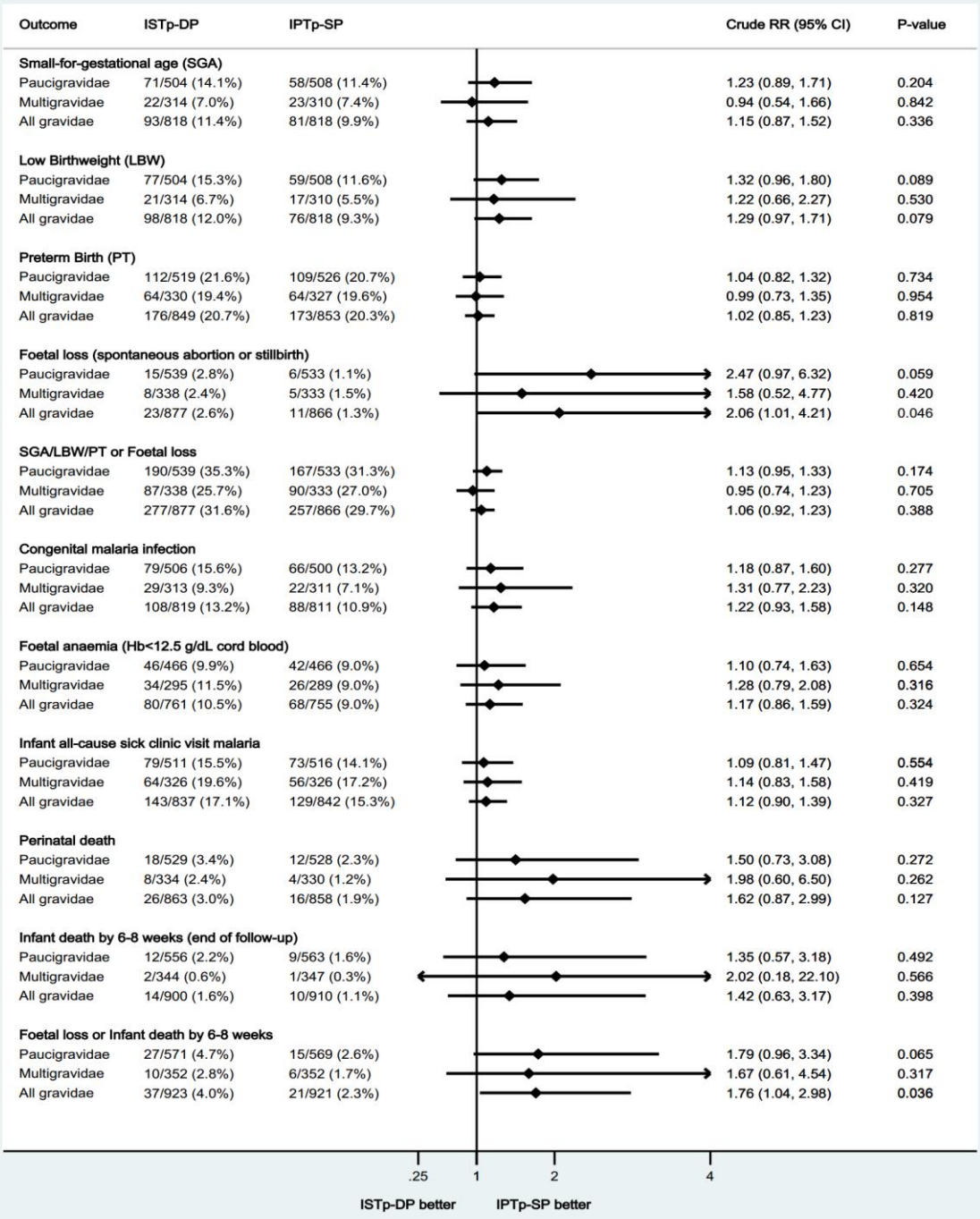


Table 4.6 Effect of ISTp-DP vs IPTp-SP on mean haemoglobin, birthweight, gestational age and mean birthweight-for gestational age Z-score (crude analysis)

	Number of women, mean (SD)		Mean difference (95% CI)	P-value
Outcome	**ISTp-DP**	**IPTp-SP**		
Maternal haemoglobin (g/dL) last scheduled visit in 3rd trimester				
Paucigravidae	408, 11.6 (1.3)	415, 11.4 (1.2)	0.12 (-0.05,0.29)	0.165
Multigravidae	262, 11.9 (1.1)	250, 11.8 (1.2)	0.09 (-0.11,0.30)	0.384
All gravidae	670, 11.7 (1.2)	665, 11.6 (1.2)	0.11 (-0.02,0.25)	0.090
Maternal haemoglobin (g/dL) at delivery				
Paucigravidae	503, 12.0 (1.6)	506, 11.7 (1.5)	0.22 (0.03, 0.41)	0.026
Multigravidae	314, 12.1 (1.5)	312, 12.1 (1.5)	-0.09 (-0.33, 0.15)	0.466
All gravidae	817, 12.0 (1.6)	818, 11.9 (1.5)	0.10 (-0.05, 0.25)	0.179
Foetal haemoglobin (g/dL) (cord blood)				
Paucigravidae	472, 15.0 (2.4)	470, 14.9 (2.1)	0.10 (-0.18, 0.39)	0.478
Multigravidae	297, 14.7 (2.3)	291, 14.9 (2.3)	-0.14 (-0.51, 0.23)	0.466
All gravidae	769, 14.9 (2.4)	761, 14.9 (2.2)	0.01 (-0.22, 0.24)	0.937

Table 4.6 Effect of ISTp-DP vs IPTp-SP on mean haemoglobin, birthweight, gestational age and mean birthweight-for gestational age Z-score (crude analysis)

	Number of women, mean (SD)		Mean difference (95% CI)	P-value
Outcome	**ISTp-DP**	**IPTp-SP**		
Mean birthweight (grams)				
Paucigravidae	504, 2859 (412)	508, 2891 (454)	-32.3 (-85.7, 21.1)	0.236
Multigravidae	314, 3020 (421)	310, 3041 (404)	-20.6 (-85.2, 44.1)	0.533
All gravidae	818, 2921 (423)	818, 2948 (442)	-27.1 (-69.0, 14.8)	0.205
Gestational age (weeks) at birth				
Paucigravidae	533, 38.0 (2.5)	532, 38.2 (2.4)	-0.15 (-0.44, 0.15)	0.327
Multigravidae	338, 38.2 (2.2)	332, 38.5 (2.0)	-0.28 (-0.59, 0.04)	0.083
All gravidae	871, 38.1 (2.4)	864, 38.3 (2.3)	-0.20 (-0.41, 0.02)	0.076
Birthweight for gestational age Z-score				
Paucigravidae	501, 0.0 (0.9)	505, 0.1 (0.9)	-0.06 (-0.18, 0.05)	0.280
Multigravidae	314, 0.3 (1.0)	309, 0.3 (0.9)	0.02 (-0.13, 0.17)	0.766
All gravidae	815, 0.1 (0.9)	814, 0.2 (1.0)	-0.03 (-0.12, 0.06)	0.540

Table 4.7 Difference in number of days between antimalarial dosing and delivery

Arm	Number of women with event	Mean (std.dev)	Difference (95% CI), p-value	
Sulphadoxine-Pyrimethamine (SP)	796	19 (11.9)	*Reference*	
Artemether-Lumefantrine (AL)	21	21 (21.0)	-2 (-6.7, 3.5), 0.537	*Reference*
Dihydroartemesinin-Piperaquine (DP)	70	17 (9.6)	2 (-0.6, 5.2), 0.127	4 (-0.9, 8.6), 0.114

Last visit prior to delivery may have been a scheduled or unscheduled visit. Peripheral malaria at delivery was defined as having parasiteamia detected by either malaria smear, RDT or PCR. Active placental malaria was defined as either acute or chronic placental infection

Table 4.8 Effect of antimalarial dosing at last visit prior to delivery on maternal peripheral parasiteamia and active placental malaria

Outcome	no/No (%) of women with events	Risk Ratio (95% CI), p-value	
Peripheral parasitaemia			
Sulphadoxine-Pyrimethamine (SP)	130/756 (17.2)	*Reference*	
Artemether-Lumefantrine (AL)	11/20 (55.0)	3.20 (2.09, 4.90), <0.001	*Reference*
Dihydroartemesinin-Piperaquine (DP)	20/67 (29.9)	1.74 (1.16, 2.59), 0.007	0.54 (0.32, 0.93), 0.027
Active placental malaria			
Sulphadoxine-Pyrimethamine (SP)	249/748 (33.3)	*Reference*	
Artemether-Lumefantrine (AL)	15/20 (75.0)	2.25 (1.72, 2.96), <0.001	*Reference*
Dihydroartemesinin-Piperaquine (DP)	29/66 (43.9)	1.32 (0.99, 1.77), 0.061	0.59 (0.40, 0.85), 0.005

Last visit prior to delivery may have been a scheduled or unscheduled visit. Peripheral malaria at delivery was defined as having parasiteamia detected by either malaria smear, RDT or PCR. Active placental malaria was defined as either acute or chronic placental infection

Table 4.9 Proportion of women reinfected by next antenatal visit after treatment with DP

Visit after booking	Number of women treated at prior visit	Number of women reinfected at current visit †	Proportion reinfected (%)
1st	291	50	17
2nd	29	8	28
3rd	28	9	32
Delivery	52	0	0
Total	400	67	17

† Peripheral blood reinfection by either light microscopy, RDT or PCR positive

Table 4.10 Adherence and tolerance of study drugs and regimen

Outcome	ISTp-DP	IPTp-SP
Number of women who did not tolerate DP or SP at least once (%)[a]		
Paucigravidae	1/351 (0.3)	0/569 (0.0)
Multigravidae	1/122 (0.8)	0/352 (0.0)
All gravidae	2/473 (0.4)	0/921 (0.0)

a. Defined as vomiting of primary and repeat dose, requiring alternative treatment with AL or parenteral antimalarials

Table 4.11 Foetal loss, perinatal and infant death among DP recipients and non-recipients in the ISTp-DP arm, compared with the IPTp-SP arm

Outcome	# with (at least one) event / # followed (%), or # events, # with (at least one) event/ # followed (%)				Risk Ratio (95% CI), p-value			
	ISTp-DP: DP-non-recipients	ISTp-DP: DP-recipients	ISTp-DP total	IPTP-SP	ISTp-DP total vs IPTp-SP	ISTp-DP non-DP-recipients vs DP-recipients	ISTp-DP non-DP-recipients vs IPTp-SP arm	ISTp-DP DP-recipients vs IPTp-SP arm
Maternal SAEs (cumulative risk [% at least 1 event])								
Paucigravidae	12/220 (5.5)	15/351 (4.3)	27/571 (4.7)	29/569 (5.1)	0.93 (0.56, 1.55), 0.7738	1.28 (0.61, 2.68), 0.5182	1.07 (0.56, 2.06), 0.8390	0.84 (0.46, 1.54), 0.5708
Multigravidae	12/230 (5.2)	4/122 (3.3)	16/352 (4.5)	16/352 (4.5)	1.00 (0.51, 1.97), 1.0000	1.59 (0.52, 4.83), 0.4121	1.15 (0.55, 2.38), 0.7112	0.72 (0.25, 2.12), 0.5519
All gravidae	24/450 (5.3)	19/473 (4.0)	43/923 (4.7)	45/921 (4.9)	0.95 (0.63, 1.43), 0.8190	1.33 (0.74, 2.39), 0.3446	1.09 (0.67, 1.77), 0.7219	0.82 (0.49, 1.39), 0.4644
Maternal deaths								
Paucigravidae	0/220 (0.0)	0/351 (0.0)	0/571 (0.0)	1/569 (0.2)	CBE[a]	CBE[a]	CBE[a]	CBE[a]
Multigravidae	0/230 (0.0)	0/122 (0.0)	0/352 (0.0)	1/352 (0.3)	CBE[a]	CBE[a]	CBE[a]	CBE[a]
All gravidae	0/450 (0.0)	0/473 (0.0)	0/923 (0.0)	2/921 (0.2)	CBE[a]	CBE[a]	CBE[a]	CBE[a]
Congenital malformations								
Paucigravidae	3/195 (1.5)	6/333 (1.8)	9/528 (1.7)	8/529 (1.5)	1.13 (0.44, 2.90), 0.8039	0.85 (0.22, 3.38), 0.8218	1.02 (0.27, 3.80), 0.9796	1.19 (0.42, 3.40), 0.7436
Multigravidae	3/217 (1.4)	1/117 (0.9)	4/334 (1.2)	1/330 (0.3)	3.95 (0.44, 35.17), 0.2179	1.62 (0.17, 15.38), 0.6756	4.56 (0.48, 43.58), 0.1874	2.82 (0.18, 44.73), 0.4621
All gravidae	6/412 (1.5)	7/450 (1.6)	13/862 (1.5)	9/859 (1.0)	1.44 (0.62, 3.35), 0.3980	0.94 (0.32, 2.76), 0.9050	1.39 (0.50, 3.88), 0.5295	1.48 (0.56, 3.96), 0.4298
Foetal Loss								
Paucigravidae	7/201 (3.5)	8/338 (2.4)	15/539 (2.8)	6/533 (1.1)	2.47 (0.97, 6.32), 0.0589	1.47 (0.54, 4.00) 0.4487	3.09 (1.05, 9.09), 0.0401	2.10 (0.74, 6.01), 0.1653
Multigravidae	6/220 (2.7)	2/118 (1.7)	8/338 (2.4)	5/333 (1.5)	1.58 (0.52, 4.77), 0.4204	1.61 (0.33, 7.85) 0.5563	1.82 (0.56, 5.88), 0.3193	1.13 (0.22, 5.74), 0.8839

Table 4.11 Foetal loss, perinatal and infant death among DP recipients and non-recipients in the ISTp-DP arm, compared with the IPTp-SP arm

	# with (at least one) event / # followed (%), or # events, # with (at least one) event/ # followed (%)				Risk Ratio (95% CI), p-value			
Outcome	ISTp-DP: DP-non-recipients	ISTp-DP: DP-recipients	ISTp-DP total	IPTP-SP	ISTp-DP total vs IPTp-SP	ISTp-DP non-DP-recipients vs DP-recipients	ISTp-DP non-DP-recipients vs IPTp-SP arm	ISTp-DP DP-recipients vs IPTp-SP arm
All gravidae	13/421 (3.1)	10/456 (2.2)	23/877 (2.6)	11/866 (1.3)	2.06 (1.01, 4.21), 0.0461	1.41 (0.62, 3.18), 0.4098	2.43 (1.10, 5.38), 0.0284	1.73 (0.74, 4.03), 0.2073
Miscarriage								
Paucigravidae	3/201 (1.5)	2/338 (0.6)	5/539 (0.9)	2/533 (0.4)	2.47 (0.48, 12.69), 0.2681	2.52 (0.43, 14.97), 0.3085	3.98 (0.67, 23.63), 0.1288	1.58 (0.22, 11.14), 0.6480
Multigravidae	2/220 (0.9)	0/118 (0)	2/338 (0.6)	1/333 (0.3)	1.97 (0.18, 21.63), 0.5790	CBE[a]	3.03 (0.28, 33.18), 0.3646	CBE[a]
All gravidae	5/421 (1.2)	2/456 (0.4)	7/877 (0.8)	3/866 (0.3)	2.30 (0.60, 8.88), 0.2253	2.71 (0.53, 13.88), 0.2323	3.43 (0.82, 14.28), 0.0905	1.27 (0.21, 7.55), 0.7957
Stillbirth								
Paucigravidae	4/198 (2.0)	6/336 (1.8)	10/534 (1.9)	4/531 (0.8)	2.49 (0.78, 7.88), 0.1217	1.13 (0.32, 3.96), 0.8470	2.68 (0.68, 10.62), 0.1601	2.37 (0.67, 8.34), 0.1786
Multigravidae	4/218 (1.8)	2/118 (1.7)	6/336 (1.8)	4/332 (1.2)	1.48 (0.42, 5.20), 0.5392	1.08 (0.20, 5.82), 0.9264	1.52 (0.38, 6.03), 0.5489	1.41 (0.26, 7.58), 0.6913
All gravidae	8/416 (1.9)	8/454 (1.8)	16/870 (1.8)	8/863 (0.9)	1.98 (0.85, 4.61), 0.1114	1.09 (0.41, 2.88), 0.8599	2.07 (0.78, 5.49), 0.1416	1.90 (0.72, 5.03), 0.1959
Infant SAEs (cumulative risk [% at least 1 event])								
Paucigravidae	5/194 (2.6)	18/330 (5.5)	23/524 (4.4)	19/527 (3.6)	1.22 (0.67, 2.21), 0.5172	0.47 (0.18, 1.25), 0.1317	0.71 (0.27, 1.89), 0.4982	1.51 (0.81, 2.84), 0.1976
Multigravidae	6/214 (2.8)	4/116 (3.4)	10/330 (3.0)	4/328 (1.2)	2.48 (0.79, 7.84), 0.1206	0.81 (0.23, 2.82), 0.7446	2.30 (0.66, 8.05), 0.1930	2.83 (0.72, 11.12), 0.1369
All gravidae	11/408 (2.7)	22/446 (4.9)	33/854 (3.9)	23/855 (2.7)	1.44 (0.85, 2.43), 0.1754	0.55 (0.27, 1.11), 0.0960	1.00 (0.49, 2.04), 0.9951	1.83 (1.03, 3.25), 0.0381
Perinatal death								
Paucigravidae	7/196 (3.6)	11/333 (3.3)	18/529 (3.4)	12/528 (2.3)	1.50 (0.73, 3.08), 0.2722	1.08 (0.43, 2.74), 0.8695	1.57 (0.63, 3.93), 0.3343	1.45 (0.65, 3.26), 0.3635

Table 4.11 Foetal loss, perinatal and infant death among DP recipients and non-recipients in the ISTp-DP arm, compared with the IPTp-SP arm

	# with (at least one) event / # followed (%), or # events, # with (at least one) event/ # followed (%)				Risk Ratio (95% CI), p-value			
Outcome	ISTp-DP: DP-non-recipients	ISTp-DP: DP-recipients	ISTp-DP total	IPTP-SP	ISTp-DP total vs IPTp-SP	ISTp-DP non-DP-recipients vs DP-recipients	ISTp-DP non-DP-recipients vs IPTp-SP arm	ISTp-DP DP-recipients vs IPTp-SP arm
Multigravidae	6/216 (2.8)	2/118 (1.7)	8/334 (2.4)	4/330 (1.2)	1.98 (0.60, 6.50), 0.2622	1.64 (0.34, 7.99), 0.5411	2.29 (0.65, 8.03), 0.1947	1.40 (0.26, 7.54), 0.6964
All gravidae	13/412 (3.2)	13/451 (2.9)	26/863 (3.0)	16/858 (1.9)	1.62 (0.87, 2.99), 0.1267	1.09 (0.51, 2.33), 0.8149	1.69 (0.82, 3.48), 0.1536	1.55 (0.75, 3.18), 0.2377
Neonatal death (by 4 weeks)								
Paucigravidae	4/192 (2.1)	6/327 (1.8)	10/519 (1.9)	8/524 (1.5)	1.26 (0.50, 3.17), 0.6207	1.14 (0.32, 3.97), 0.8425	1.36 (0.42, 4.48), 0.6083	1.20 (0.42, 3.43), 0.7313
Multigravidae	2/212 (0.9)	0/116 (0)	2/328 (0.6)	0/326 (0)	CBE[a]	CBE[a]	CBE[a]	CBE [a]
All gravidae	6/404 (1.5)	6/443 (1.4)	12/847 (1.4)	8/850 (0.9)	1.51 (0.62, 3.66), 0.3675	1.10 (0.36, 3.37), 0.8723	1.58 (0.55, 4.52), 0.3954	1.44 (0.50, 4.12), 0.4978
Infant death (by 6 to 8 weeks)								
Paucigravidae	4/213 (1.9)	8/343 (2.3)	12/556 (2.2)	9/563 (1.6)	1.35 (0.57, 3.18), 0.4920	0.81 (0.25, 2.64), 0.7207	1.17 (0.37, 3.77), 0.7867	1.46 (0.57, 3.75), 0.4323
Multigravidae	2/224 (0.9)	0/120 (0)	2/344 (0.6)	1/347 (0.3)	2.02 (0.18, 22.15), 0.5659	CBE[a]	3.10 (0.28, 33.97), 0.3547	CBE, 0.5561
All gravidae	6/437 (1.4)	8/463 (1.7)	14/900 (1.6)	10/910 (1.1)	1.42 (0.63, 3.17), 0.3982	0.79 (0.28, 2.27), 0.6680	1.25 (0.46, 3.42), 0.6643	1.57 (0.62, 3.96), 0.3365
Foetal loss or infant death								
Paucigravidae	11/220 (5.0)	16/351 (4.6)	27/571 (4.7)	15/569 (2.6)	1.79 (0.96, 3.34), 0.0649	1.10 (0.52, 2.32), 0.8088	1.90 (0.88, 4.06), 0.0998	1.73 (0.87, 3.45), 0.1207
Multigravidae	8/230 (3.5)	2/122 (1.6)	10/352 (2.8)	6/352 (1.7)	1.67 (0.61, 4.54), 0.3173	2.12 (0.46, 9.84), 0.3364	2.04 (0.72, 5.80), 0.1811	0.96 (0.20, 4.70), 0.9616
All gravidae	19/450 (4.2)	18/473 (3.8)	37/923 (4.0)	21/921 (2.3)	1.76 (1.04, 2.98), 0.0361	1.11 (0.59, 2.09), 0.7471	1.85 (1.01, 3.41), 0.0478	1.67 (0.90, 3.10), 0.1052

CBE=RR and 95% CI cannot be estimated

4.2 Discussion

ISTp has been compared with IPTp-SP in two prior studies, but ours is the first to use DP and the first to evaluate the effects of ISTp among women that are protected by LLINs in areas with high malaria transmission and a high prevalence of parasite SP-resistant genotypes. Despite the high levels of resistance, ISTp-DP was not superior to the standard IPTp-SP regimen and was associated with more malaria at delivery, more LBW (p=0.067), and more foetal loss. Although the relative increase in malaria risk was modest, this resulted in an additional 8 out every 100 pregnancies being affected. These results suggest that ISTp-DP may not be a suitable alternative strategy to replace IPTp-SP in areas with high SP-resistance and may even predispose to unfavourable pregnancy outcomes.

Our efficacy findings are consistent with two previous non-inferiority trials conducted in areas in West Africa (338, 339). This consistency is surprising because of the marked geographic differences in prevailing SP resistance, which is high in Malawi but low in West Africa. In both West African studies, ISTp was non-inferior to IPTp-SP in the reduction in LBW among paucigravidae, though mean birthweights were higher in the IPTp-SP recipients than those receiving ISTp with amodiaquine-artesunate (p=0.06) (338) or AL (p=0.04) (339). Additionally, the incidence of clinical malaria was higher in the ISTp-AL arm compared to IPTp-SP. This was not observed in our trial, possibly reflecting the longer post-treatment prophylaxis with DP compared to AL.

The lack of superiority of ISTp-DP in the current trial may result from the ineffectiveness of ISTp as a strategy or from the continued effectiveness of IPTp-SP despite prevalent SP resistance. In our study area in Malawi, SP resistance was high, with 99% of parasites harbouring the “quintuple mutant” haplotype and 1.5% (209) to 4% (340) harbouring the additional *pfdhps*-A581G mutation, which classifies it as a high, but not yet super-resistant, area (341); therefore it is likely that IPTp-SP continued to provide some benefits, as has been observed in settings with similar parasite

populations (342, 343). Another factor likely contributing to continued effectiveness of IPTp-SP is our use of the frequent dosing regimen (3) now recommended by WHO, which may mitigate the shortening of post-treatment prophylaxis resulting from SP resistance (343). It would also be of interest to further explore whether SP, which also has broad antimicrobial activity, may have conferred additional protection from other pathogens (344).

Further to the surprising lack of difference in the overall efficacy of ISTp irrespective of significant differences in SP resistance with sites in West Africa presented above, transmission intensity may prove to be a significant determinant of the efficacy of ISTp owing to both sites having high transmission despite differences in SP resistance. With close to half of the women experiencing at least one episode of infection after enrolment, the evidence suggests an overall high transmission setting. It may be postulated that within a high transmission setting, a higher probability of infection occurs due to an increased frequency of exposure to infective bites, culminating in pregnant women having a higher risk of being infected and developing a significant biomass of parasites that lead to adverse pregnancy outcomes before they are diagnosed by RDT at the next antenatal visit. This is evident in the differences in the site specific primary malaria endpoints between two sites with moderate to high transmission and the one site with low transmission. It is likely that preceding studies utilising screening strategies, such as the Mass Screening and Treatment trials (345, 346), may have been informative in the consideration of screening in pregnancy to highlight the possible role of transmission intensity. Other than this, there would have very limited plausibility for the expected impact in pregnant populations. This is because the effect of the phenomenon of placental sequestration and gravidity dependent immunity would not have been observable in non-pregnant populations. In addition, screening and treatment approaches in non-pregnant populations are primarily targeted toward transmission reduction by detecting and treating the parasite reservoir as opposed to clinical outcomes which are of primary importance in pregnancy interventions

Without any significant differences in the time between receiving DP, AL and SP at the last visit prior to delivery, the findings on the risk of peripheral and active placental malaria are rather surprising. The quintuple resistance genotype conferring SP resistance would be expected to reduce the treatment or prophylactic efficacy of SP (347) compared to DP and AL. As such, it would be expected that receipt of SP would be associated with a higher risk of peripheral and placental parasiteamia. Our findings do not support this expectation. Parasite density is known to be a predictor of parasitological failure in treatment studies (348). The use of DP and AL was indicated in patent or symptomatic infection, which would be characterised by higher parasite densities than with asymptomatic infection where SP was administered. It is therefore conceivable that parasite failure at delivery was due to differences in parasite densities at the time of administration of the antimalarial. Conversely, the results also suggest that SP continues confer a beneficial antimalarial effect despite high resistance levels resulting in low levels of parasiteamia and lower risks of parasite failure at delivery, likely through a mechanism of suppression of infection through the frequent doses over the course of pregnancy (349).

ISTp-DP may have been ineffective owing to a failure to detect low-level parasitaemia. Conceptually, ISTp is intended to prevent both existing infections from progressing as well as new infections from occurring for up to 6 weeks after each DP course. The majority of antenatal infections were undetected by RDTs, thereby allowing sub-patent (RDT-negative, PCR-positive) infections to persist in the placenta. This was particularly noticeable among multigravidae, who tend to have lower density infections when infected. The overall sensitivity of RDT to detect PCR-positive infections during pregnancy was 44.9% in paucigravidae (51.6% in G1, 35.8% in G2) and 29.2% in multigravidae (Figure 5.3, section 5.2.2). This may explain the somewhat greater difference between treatment arms in malaria prevalence at delivery in multigravidae than primigravidae. The greater predisposition for active placental malaria at delivery than past or chronic infection may most likely be explained by the persistence of infections missed by RDT screening at the final visit prior to

delivery in the IST arm. By not treating these infections, they were left to either progress unfettered in the peripheral blood and seed in the placenta or were already present in the placenta and not cleared by treatment. This is in direct contrast to the SP arm where the routine administration of SP at the last visit would have cleared any present infections in the peripheral blood or placenta prior to delivery.

It is unlikely that suboptimal dosing or sub-therapeutic levels of DP would have contributed to the non-superior performance of ISTp-DP: each dose was supervised and there is no evidence that pregnancy alters the pharmacokinetics of DP to a degree that is requires dose adjustment (350). The same DP regimen was shown to be highly effective (PCR-corrected success rate: 98.8%) in a concurrent treatment trial conducted by the same team in this area using the same batch (172, 351). The progressive increase in the risk of reinfection following treatment with dihydroartemisinin-piperaquine may have arisen as a result of women from transmission hot-spots presenting with frequent repeated infections. However, it cannot be determined whether the observed reinfections were due to reinfection or recrudescence. The lack of reinfection being detected at delivery could be attributed to the brief time frame between the last antenatal visit when the dose of DP was received and delivery, reflecting the acute treatment efficacy of DP.

The higher rate of foetal loss in the ISTp-DP arm (2.6% vs 1.3%) may reflect the ineffectiveness of ISTp to prevent malaria. There was no indication that this was related to DP toxicity as the risk was highest among pregnancies that had never received DP (3.1% vs 2.2%). Consistent with the recent 4-arm treatment trial comparing the 4 fixed dose ACTs in the case-management of malaria in pregnancy (351), DP was well tolerated. This is important as almost all RDT-positive women in our trial were asymptomatic and tolerance can be a major factor determining adherence.

ISTp is a labour intensive strategy, but it was highly acceptable to both patients and clinic staff. This finding was documented through a separate qualitative sub-study using in-depth interviews and focus-group discussions (Almond D, personal communications), corroborating

findings from similar acceptability studies in Ghana (352, 353). Although more frequent blood sampling was involved, women appreciated its importance and the fact that they could be shown the RDT test results. The venous sampling at antenatal booking was deemed more convenient than repeated finger pricks as it allowed health workers to tests for malaria, anaemia, syphilis and HIV testing with a single blood draw.

This study has a number of limitations. We did not conduct genotyping for SP resistance in individual women to determine the role of the *dhps*-A581G mutation. Furthermore, no molecular assessments were undertaken to differentiate recrudescence from reinfections needed to determine what fraction of infections in the IPTp-SP result from recrudescence due to sub-optimal clearance by SP.

IPTp-DP was not superior to the existing IPTp-SP regimen in this area with high SP resistance in southern Malawi. These results should be equally relevant to other areas in east and southern Africa with similar or lower levels of parasite SP resistance. In super-resistant settings, where alternative drugs that can replace SP for IPTp are lacking, ISTp-DP could be a potential interim solution. The identification for alternative drugs to replace SP remains a high research priority for the control of malaria in pregnancy before levels of SP resistance render IPTp-SP fully ineffective. Further considerations to the discussion of these findings and the implications thereof are presented in the overall discussion in Chapter 7.

5 CHAPTER FIVE: THE EFFECT OF ASYMPTOMATIC PARASITAEMIA ON PREGNANCY OUTCOMES.

5.1 Statistical consideration and analysis

The present analysis was based on a cohort of women from the ISTp arm of the prior presented clinical trial, the methods of which have been previously presented in Chapter 3. The objective of this analysis was to determine whether the lack of a superior performance by ISTp may have been partly attributable to low density peripheral blood infections missed by RDT but detectable by PCR over the course of the antenatal period. Specifically the analysis was undertaken to determine:

1. The effect of timing of sub-RDT infections on the risk of maternal anaemia, placental malaria and adverse live birth outcomes,
2. The effect of frequency of sub-RDT infections on the risk of maternal anaemia, placental malaria and adverse live birth outcomes, and
3. The effect of patent RDT asymptomatic parasitaemia on maternal anaemia, placental malaria and adverse live birth outcomes.

5.1.1 Exposures

For the purposes of this analysis, women were classified into the following infection statuses based on their overall experience of malaria infection over the course of their pregnancies, including at enrolment:

1) No parasitaemia; RDT and PCR negative at all visits,
2) Only asymptomatic sub-RDT parasitaemia; RDT negative but PCR positive at least once, or were otherwise without parasitaemia (RDT and PCR negative) at any given visit
3) Only asymptomatic RDT patent parasitaemia; RDT and PCR positive only at least once, or were otherwise without parasitaemia (RDT and PCR negative) at any given visit, and

4) Mixed infection experience; experienced at least one episode of asymptomatic sub-RDT and one episode of asymptomatic patent and one episode of clinical malaria or any combination of two such infections, through the course of pregnancy.

RDT results were interpreted positive if the control line was visible and either the PAN (pLDH), Pf (HRP2) or both lines were visible following the manufacturer's instructions on test conduct.

The timing of infection was classified as second trimester only (corrected gestational age 13 – 26 weeks), third trimester only (corrected gestational age > 26 weeks) or both. The frequency of infection was defined as either 1 episode or 2 or more episodes.

5.1.2 Outcomes

The following outcomes from the main trial were used to evaluate the effect of sub-RDT parasitaemia:

1. any maternal anaemia (Hb < 11.0 g/dl),
2. mean haemoglobin concentration at final scheduled antenatal visit,
3. any placental malaria (active or chronic placental malaria),
4. small for gestational age (SGA, corrected birthweight for corrected gestational age at birth < 10^{th} percentile),
5. preterm birth (PTB, corrected gestational age at delivery < 37 weeks)
6. mean gestational age (corrected)
7. low birth weight (LBW, corrected birthweight at delivery < 2500 grams)
8. mean birthweight (corrected)
9. any adverse live birth outcomes (composite of SGA, PTB and LBW)

5.1.3 Analyses

Analysis was performed with STATA version 13.0 software (Stata Corp., College Station, TX, USA). Categorical baseline characteristics were compared using the χ^2 and Fisher's exact tests.

Continuous variables were compared using the t-test. The diagnostic sensitivity of peripheral blood RDT was calculated with the user written programme stb59 sbe36_1 (http://www.stata.com/stb/stb59). Univariate and multivariate analysis were conducted using generalized linear regression models with a log link, Poisson distribution, and robust variance estimator (354, 355) to determine the crude and adjusted risk ratios between exposure status and outcome of interest, stratified by gravidity and overall. Crude and adjusted incidence rate ratios were calculated using Poisson regression models with robust standard errors as recommended by Cameron and Trivedi (356) and person years of follow-up as an offset. The t-test was used to determine the mean difference in continuous outcomes. All analyses were additionally stratified by gravidity. Two sided P-values were used and confidence intervals calculated at the 95% level. Statistical significance was set at $P \leq 0.05$.

Women with mixed infections were excluded from the analysis. This was because the occurrence of several varying degrees of infections would not enable a clear distinction of the effect of a particular infection on its own, which was the objective of the analysis, with particular emphasis on determining the effect of asymptomatic sub-RDT infections that are postulated to be responsible for the less than superior performance of IST by being missed by the screening tool.

5.2 Results

5.2.1 Study population and baseline characteristics

452 (49.0%) of the women enrolled in the ISTp arm were included in the analysis (Figure 5.1) with data on haemoglobin at last scheduled antenatal visit and delivery visit available for 371 (40.2%) and 443 (48.0%) respectively. 51% of the women enrolled in the ISTp arm in the main trial were excluded due to having mixed infection experiences.

5.2.2 Malaria infection and the diagnostic performance of peripheral blood RDT

Less than half of the women in the IST arm prior to exclusion had evidence of malaria infection by either RDT or PCR (451/923, 48.9%). Amongst the cohort of women included in this analysis, any infection by RDT or PCR was 46.2%, n= 452. Slightly under a third of all the women included in the analysis (29.0%, n = 452) were infected with malaria at enrolment by RDT (the corresponding figure in the overall sample of women in the ISTp arm was 318/922 (34.5%). The prevalence of infection by PCR at the same time point in this cohort was 44.5% (whilst the corresponding prevalence in the overall women from the IST arm was 44.3%, n=903). Malaria infection prevalence by both RDT and PCR were higher in paucigravidae at all visits but the prevalence of sub-RDT infection was consistently higher in multigravidae compared to paucigravidae (Figure 5.2).

PCR was used as the gold standard against which the diagnostic sensitivity of the malaria RDT was assessed. The overall diagnostic sensitivity of peripheral blood RDT was 66.8% (95% C.I. 62.0, 71.4%). The sensitivity was higher in primigravidae than secundi- and multigravidae (85.8%, 95% C.I. 79.8, 90.6% vs 57.8%, 95% C.I. 48.0, 67.2% vs 45.6%, 95% C.I. 36.3, 55.2% respectively) and this trend remained through pregnancy (Figure 5.3).

5.2.3 Effect of asymptomatic parasitaemia on maternal outcomes

5.2.3.1 Anaemia

Asymptomatic sub-RDT parasitaemia was not associated with an increased significantly increased risk of developing maternal anaemia compared to women who never experienced any parasitaemia (39.0 vs 37.4%, RR 1.04, 95% C.I. 0.78, 1.39, p-value = 0.779). This effect did not change after stratifying by gravidity (Table 5.2) and was not different by frequency (Table 5.3) and timing of sub-RDT infection (Table 5.4).

No notable effect in timing of sub-RDT parasitaemia on the incidence of maternal anaemia (Tables 5.5 and 5.6) or difference in mean haemoglobin concentration at last scheduled antenatal visit (Tables 5.7 and 5.8) was observed.

Figure 5.1 Participant flow chart

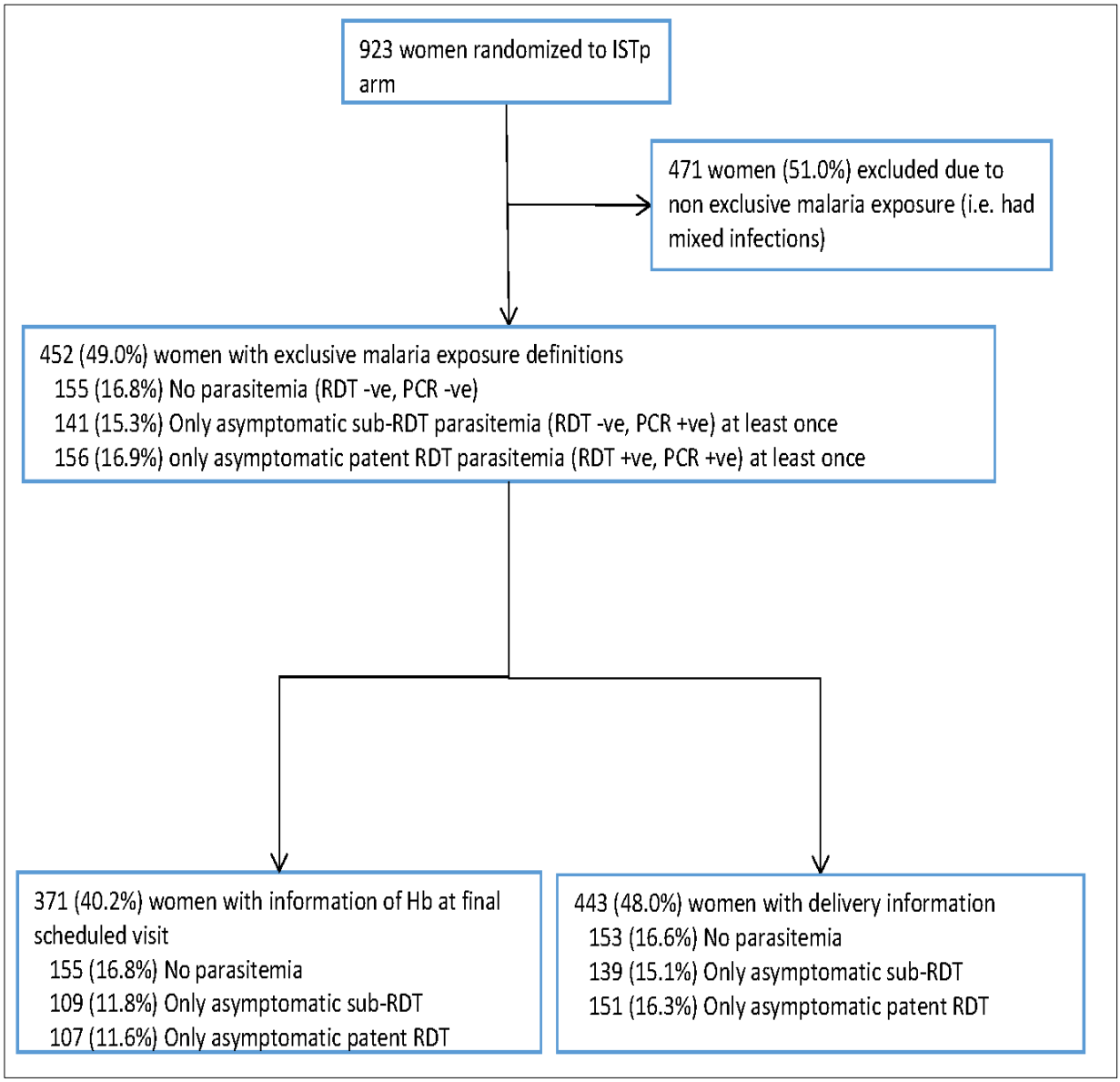


Table 5.1 Baseline patient characteristics overall and by parasitaemia class

Characteristic		**Overall** (N=452)	**No parasitaemia** (n=155)	**Only asymptomatic sub-RDT parasitaemia** (n=141)	**Only asymptomatic RDT Patent parasitaemia** (n=156)
Age (years) mean (sd)		22.9 (5.2)	24.1 (5.5)	23.9 (4.8)	20.8 (4.6)
Gestational age (corrected, in weeks) mean (sd)		20.6 (3.0)	19.9 (2.4)	21.0 (3.0)	20.9 (3.5)
Gravidity *Paucigravidae (%)*		56.0	45.2	49.7	72.4
Own bednet (%)		18.1	20.7	12.8	20.5
Haemoglobin (g/dl) mean (sd)		11.1 (1.5)	11.6 (1.4)	11.5 (1.3)	10.4 (1.5)
Anaemia (Hb < 11.0 g/dl) (%)		40.0	27.1	29.1	62.8
Socioeconomic					
Residence					
	Rural	99.3	99.4	99.3	99.4
	Urban	0.2	0.0	0.0	0.6
	Peri-urban	0.5	0.6	0.7	0.0
Income category					
	Low	31.9	28.4	36.9	31.9
	Medium	35.5	32.3	36.1	35.5
	High	32.6	39.4	27.0	32.6

Figure 5.2 Malaria prevalence across antenatal visits stratified by gravidity

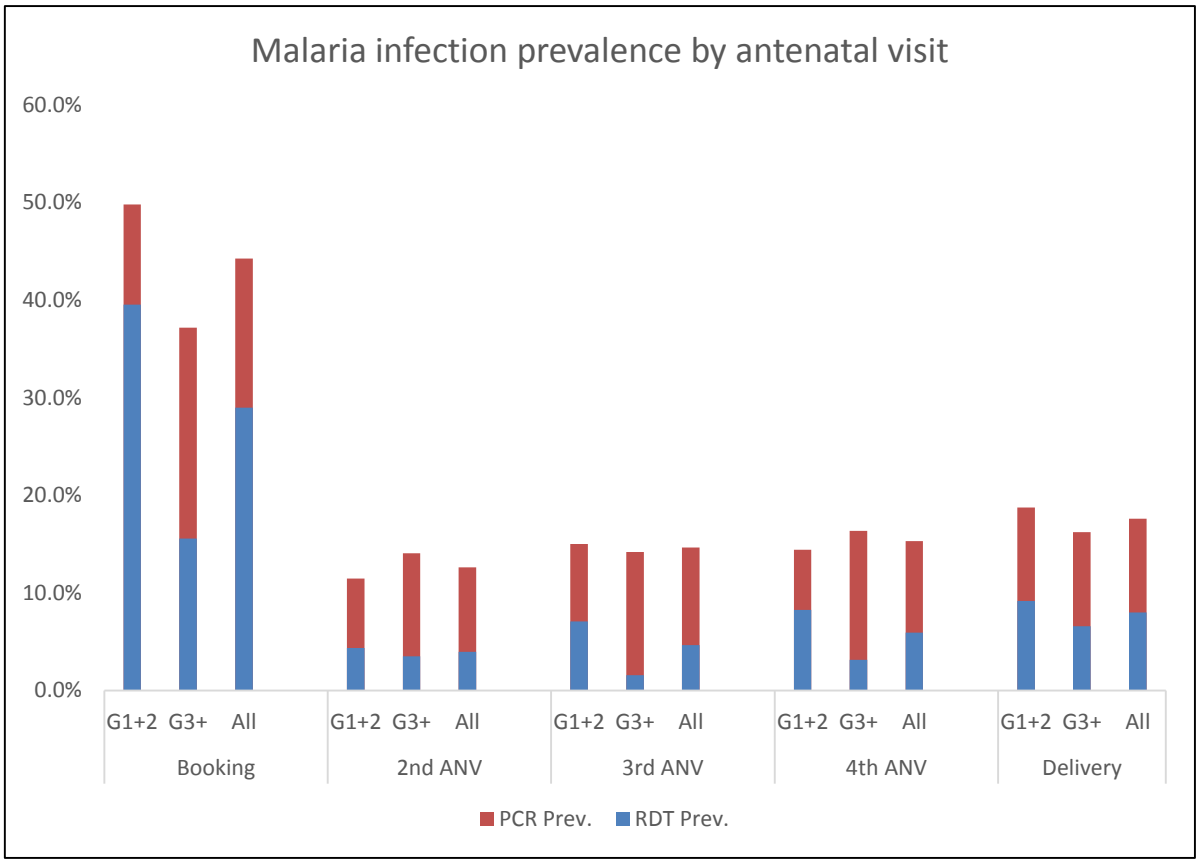
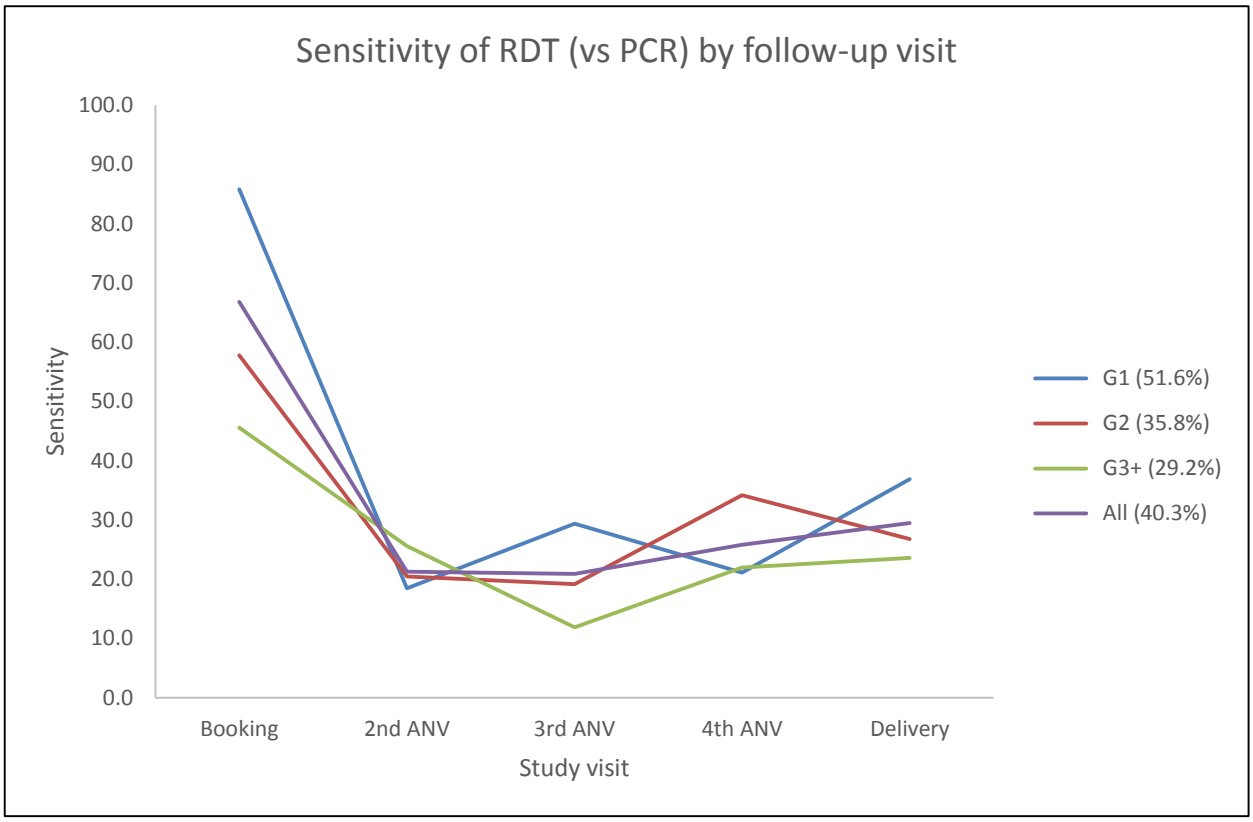


Figure 5.3 RDT sensitivity by study visit and gravidity



After adjustment for covariates, there was a significantly increased incidence of maternal anaemia with 2 or more episodes of sub-RDT infection in multigravidae (1,511.1 vs 952.2/1,000 person-years, IRR 2.17, 95% C.I. 1.12, 4.21, p-value = 0.021) (Table 5.10).

In contrast to sub-RDT infections, asymptomatic patent RDT parasitaemia significantly increased the risk of any maternal anaemia (67.3 vs 37.4%, RR 1.80, 95% C.I. 1.43, 2.27, p-value < 0.001). This effect was mostly apparent in paucigravidae (74.3 vs 44.3%, RR 1.68, 95% C.I. 1.26, 2.23, p-value < 0.001) and marginally significant in multigravidae (48.8 vs 31.8%, RR 1.54, 95% C.I. 0.99, 2.38, p-value = 0.054). There was an increased incidence of maternal anaemia with asymptomatic

patent RDT parasitaemia (Table 5.6) in both gravidity strata and overall, but this was lost after adjustment for covariates. Paucigravidae with asymptomatic patent RDT parasitaemia had a significantly lower mean haemoglobin at the last scheduled antenatal visit (11.8 vs 11.3g/dl, mean difference 0.44, 95% C.I. 0.04, 0.84, p-value = 0.033) (Table 5.7).

5.2.3.2 Placental malaria

Asymptomatic sub-RDT parasitaemia was associated with an almost two-fold increased risk of any placental malaria at delivery (38.8 vs 20.4%, RR 1.90, 95% C.I. 1.28, 2.82, p-value = 0.002) (Table 5.11), which was consistent both in pauci- and multigravidae (45.2 vs 23.4%, RR 1.93, 95% C.I. 1.14, 3.25, p-value = 0.014 and 32.8 vs 17.8%, RR 1.84, 95% C.I. 1.01, 3.37, p-value = 0.046 respectively) though this was statistically non-significant in multigravidae after adjusting for covariates. The strength of this association was evident with infection during the third trimester, further increased if infection occurred in the second and third trimester, and with 2 or more episodes of infection (Tables 5.12 and 5.13).

Asymptomatic patent RDT infection was also significantly associated with a more than two and a half fold higher risk of placental malaria overall (55.6 vs 20.4%, RR 2.72, 95% C.I. 1.90, 3.91,p-value < 0.001) and by gravidity. There was strong evidence of this increased overall risk even after adjustment for covariates.

5.2.4 Effect of asymptomatic parasitaemia on live birth fetal outcomes

5.2.4.1 Composite adverse live birth outcome (LBW/SGA/PTB)

The risk of any adverse live birth outcome was significantly increased by asymptomatic sub-RDT parasitaemia (26.5 vs 12.8%, RR 2.06, 95% C.I. 1.23, 3.42, p-value = 0.005; Table 5.14). Two or more episodes of asymptomatic sub-RDT parasitaemia were associated with a significantly increased risk of composite adverse live birth outcome in paucigravidae whilst only having one episode conferred a significantly elevated risk in multigravidae (Table 5.15). By timing, asymptomatic sub-

RDT infection was associated with higher risk of composite adverse live birth outcome when occurring in the second or third trimester (Table 5.16).

Asymptomatic patent RDT parasitaemia was associated with a close to 3 fold increased risk of any adverse live birth outcome, though the risk was higher in multigravidae than paucigravidae (33.3 vs 8.5%, RR 3.90,95% C.I. 1.70, 8.95, p-value = 0.001 and 38.8 vs 18.2, RR 2.14, 95% C.I. 1.21, 3.77, p-value = 0.009 respectively) (Table 5.14).

5.2.4.2 Small for gestational age

There were no evident significant associations of small for gestational age any asymptomatic infection overall or when stratified by gravidity (Table 5.17). Neither the timing nor frequency of asymptomatic sub-RDT infection had a significant bearing on the risk of small for gestational age even when stratified by gravidity (Tables 5.18 and 5.19).

5.2.4.3 Low birthweight

Asymptomatic sub-RDT parasitaemia did not increase the risk of low birthweight (Table 5.20) and this was not amended by timing or frequency amongst paucigravidae or multigravidae (Tables 5.21 and 5.22). Furthermore, there was no statistically significant difference in mean birthweight with women who never experienced any parasitaemia (Tables 5.23, 5.24 and 5.25).

Asymptomatic patent RDT infection was associated with a more than 2 fold risk of low birthweight (19.1 vs 6.5%, RR 2.96, 95% C.I. 1.44, 6.06, p-value =0.003). Babies born to women without any infection over the course of pregnancy had a significantly higher mean birthweight by 172.2 grams compared to babies born to women with at least 1 asymptomatic patent malaria infection only through follow-up (3,010.3 vs 2,838.1g, 95% C.I. 75.5, 268.8, p-value < 0.001).

Table 5.2 Risk of any maternal anaemia (Hb <11.0g/dl) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with at least one event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1-2						†	
No parasitaemia (reference)	70	31	31/70 (44.3)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	70	28	28/70 (40.0)	0.90 (0.61, 1.33)	0.609	0.92 (0.54, 1.54)	0.740
Only asymptomatic RDT Patent parasitaemia	113	84	84/113 (74.3)	1.68 (1.26, 2.23)	< 0.001	0.98 (0.64, 1.52)	0.939
G3+						†	
No parasitaemia (reference)	85	27	27/85 (31.8)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	71	27	27/71 (38.0)	1.19 (0.78, 1.84)	0.414	1.14 (0.66, 1.99)	0.635
Only asymptomatic RDT Patent parasitaemia	43	21	21/43 (48.8)	1.54 (0.99, 2.38)	0.054	0.94 (0.51, 1.73)	0.846
All						‡	
No parasitaemia (reference)	155	58	58/155 (37.4)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	141	55	55/141 (39.0)	1.04 (0.78, 1.39)	0.779	1.00 (0.69, 1.46)	0.976
Only asymptomatic RDT Patent parasitaemia	156	105	105/156 (67.3)	1.80 (1.43, 2.27)	< 0.001	0.97 (0.69, 1.36)	0.865

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.3 Risk of any maternal anaemia (Hb <11.0g/dl) by number of episodes of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with at least one event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	70	31	31/70 (44.3)	-	-	-	-
1 episode	50	24	24/50 (48.0)	1.08 (0.73, 1.60)	0.686	0.99 (0.80, 1.22)	0.901
2+ episodes	20	4	4/20 (20.0)	0.45 (0.18, 1.13)	0.089	0.64 (0.41, 0.99)	0.045
G3+						†	
No parasitaemia (reference)	85	27	27/85 (31.8)	-	-	-	-
1 episode	47	18	18/47 (38.3)	1.21 (0.75, 1.95)	0.445	0.99 (0.75, 1.32)	0.964
2+ episodes	24	9	9/24 (37.5)	1.18 (0.64, 2.16)	0.591	1.65 (0.92, 2.98)	0.095
All						‡	
No parasitaemia (reference)	155	58	58/155 (37.4)	-	-	-	-
1 episode	97	42	42/97 (43.3)	1.16 (0.85, 1.57)	0.350	0.99 (0.83, 1.18)	0.870
2+ episodes	44	13	13/44 (29.5)	0.79 (0.48, 1.30)	0.355	1.08 (0.74, 1.59)	0.689

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.4 Risk of any maternal anaemia (Hb <11.0g/dl) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with at least one event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	70	31	31/70 (44.3)	-	-	-	-
Second trimester only	31	11	11/31 (35.5)	0.80 (0.47, 1.38)	0.424	0.85 (0.65, 1.10)	0.211
Third trimester only	32	14	14/32 (43.8)	0.99 (0.62, 1.59)	0.960	0.99 (0.76, 1.30)	0.947
Second and Third trimester	7	3	3/7 (42.9)	0.97 (0.39, 2.37)	0.943	0.87 (0.66, 1.16)	0.353
G3+						†	
No parasitaemia (reference)	85	27	27/85 (31.8)	-	-	-	-
Second trimester only	23	8	8/23 (34.8)	1.09 (0.58, 2.08)	0.782	1.10 (0.69, 1.76)	0.683
Third trimester only	30	14	14/30 (46.7)	1.47 (0.90, 2.41)	0.127	1.07 (0.78, 1,47)	0.681
Second and Third trimester	18	5	5/18 (27.8)	0.87 (0.39, 1.96)	0.745	1.47 (0.68, 3.21)	0.532
All						‡	
No parasitaemia (reference)	155	58	58/155 (37.4)	-	-	-	-
Second trimester only	54	19	19/54 (35.2)	0.94 (0.62, 1.43)	0.772	0.94 (0.74, 1.20)	0.615
Third trimester only	62	28	28/62 (45.2)	1.21 (0.86, 1.70)	0.281	1.02 (0.83, 1.25)	0.871
Second and Third trimester	25	8	8/25 (32.0)	0.86 (0.47, 1.57)	0.614	1.16 (0.73, 1.86)	0.530

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.5 Incidence of maternal anaemia (Hb <11.0g/dl) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia level	Number of women	Total number of events	Total person-years follow-up	Incidence rate (/1,000 pyrs)	Crude incidence rate ratio (95% C.I)	P-value	Adjusted incidence rate ratio (95% C.I)	P-value
G1-2							†	
No parasitaemia (reference)	70	49	33.98	1,442.0	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	70	41	32.45	1,263.5	0.88 (0.56, 1.37)	0.565	0.86 (0.67, 1.10)	0.232
Only asymptomatic RDT Patent parasitaemia	113	115	51.80	2,220.1	1.54 (1.08, 2.20)	0.018	0.82 (0.66, 1.02)	0.082
G3+							†	
No parasitaemia (reference)	85	40	42.01	952.15	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	71	46	31.85	1,444.27	1.52 (0.90, 2.55)	0.115	1.36 (0.95, 1.94)	0.092
Only asymptomatic RDT Patent parasitaemia	43	27	18.25	1,479.45	1.55 (0.93, 2.60)	0.093	0.93 (0.67, 1.27)	0.639
All							‡	
No parasitaemia (reference)	155	89	75.99	1,171.2	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	141	87	64.30	1,353.0	1.16 (0.82, 1.63)	0.411	1.07 (0.85, 1.35)	0.562
Only asymptomatic RDT Patent parasitaemia	156	142	70.05	2,027.1	1.73 (1.30, 2.31)	< 0.001	0.87 (0.72, 1.06)	0.178

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.6 Incidence of maternal anaemia (Hb <11.0g/dl) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia level	**Number of women**	**Total number of events**	**Total person-years follow-up**	**Incidence rate (/1,000 pyrs)**	**Crude incidence rate ratio (95% C.I)**	**P-value**	**Adjusted incidence rate ratio (95% C.I)**	**P-value**
G1+2							†	
No parasitaemia (reference)	70	49	33.98	1,442.02	-	-	-	-
Second trimester only	31	16	14.42	1,109.57	0.77 (0.42, 1.42)	0.403	0.84 (0.60, 1.18)	0.314
Third trimester only	32	20	14.89	1,343.18	0.93 (0.54, 1.60)	0.797	0.87 (0.65, 1.16)	0.348
Second and Third trimester	7	5	3.13	1,597.44	1.11 (0.41, 2.96)	0.841	0.88 (0.58, 1.35)	0.573
G3+							†	
No parasitaemia (reference)	85	40	42.01	952.15	-	-	-	-
Second trimester only	23	14	10.59	1,322.00	1.39 (0.60, 3.23)	0.446	1.42 (0.78, 2.58)	0.251
Third trimester only	30	22	12.75	1,725.49	1.81 (1.02, 3.21)	0.041	1.12 (0.81, 1.56)	0.482
Second and Third trimester	18	10	8.51	1,175.09	1.23 (0.50, 3.06)	0.651	2.02 (0.86, 4.79)	0.109
All							‡	
No parasitaemia (reference)	155	89	75.99	1,171.2	-	-	-	-
Second trimester only	54	30	25.00	1,200.0	1.02 (0.61, 1.71)	0.927	1.06 (0.75, 1.50)	0.749
Third trimester only	62	42	27.64	1,519.5	1.30 (0.87, 1.92)	0.196	0.98 (0.77, 1.24)	0.856
Second and Third trimester	25	15	11.65	1,287.6	1.10 (0.56, 2.17)	0.784	1.50 (0.88, 2.56)	0.135

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.7 Difference in mean Haemoglobin at last scheduled antenatal visit by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean Hb at last scheduled antenatal visit** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	70	11.8 (1.20)	-	-
Only asymptomatic sub-RDT parasitaemia	54	11.9 (1.34)	-0.10 (-0.55, 0.35)	0.655
Only asymptomatic RDT Patent parasitaemia	78	11.3 (1.28)	0.44 (0.04, 0.84)	0.033
G3+				
No parasitaemia (reference)	85	11.9 (1.12)	-	-
Only asymptomatic sub-RDT parasitaemia	55	11.8 (1.21)	0.07 (-0.33, 0.46)	0.743
Only asymptomatic RDT Patent parasitaemia	29	12.1 (1.14)	-0.25 (-0.73, 0.23)	0.308
All				
No parasitaemia (reference)	155	11.8 (1.16)	-	-
Only asymptomatic sub-RDT parasitaemia	109	11.9 (1.27)	-0.01 (-0.31, 0.28)	0.933
Only asymptomatic RDT Patent parasitaemia	107	11.6 (1.29)	0.28 (-0.02, 0.58)	0.063

Table 5.8 Difference in mean Hb at last scheduled antenatal visit by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean gestational age at delivery** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	70	11.8 (1.20)	-	-
Second trimester only	23	12.1 (1.34)	-0.28 (-0.87, 0.31)	0.349
Third trimester only	27	11.8 (1.24)	-0.02 (-0.56, 0.53)	0.945
Second and Third trimester	4	11.4 (2.14)	0.35 (-0.93, 1.64)	0.586
G3+				
No parasitaemia (reference)	85	11.9 (1.12)	-	-
Second trimester only	15	12.1 (1.18)	-0.18 (-0.81, 0.45)	0.577
Third trimester only	24	11.7 (1.31)	0.22 (-0.31, 0.76)	0.411
Second and Third trimester	16	11.8 (1.12)	0.06 (-0.55, 0.67)	0.850
All				
No parasitaemia (reference)	155	11.8 (1.16)	-	-
Second trimester only	38	12.1 (1.26)	-0.22 (-0.64, 0.20)	0.299
Third trimester only	51	11.7 (1.26)	0.10 (-0.27, 0.48)	0.589
Second and Third trimester	20	11.8 (1.32)	0.09 (-0.46, 0.64)	0.751

Table 5.9 Difference in mean Hb last scheduled antenatal visit by frequency of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean gestational age at delivery** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	70	11.8 (1.20)	-	-
1 episode	40	11.8 (1.36)	-0.04 (-0.54, 0.45)	0.872
2+ episodes	14	12.1 (1.30)	-0.28 (-0.99, 0.43)	0.433
G3+				
No parasitaemia (reference)	85	11.9 (1.12)	-	-
1 episode	34	11.9 (1.28)	-0.03 (-0.50, 0.44)	0.906
2+ episodes	21	11.7 (1.11)	0.22 (-0.32, 0.76)	0.427
All				
No parasitaemia (reference)	155	11.8 (1.16)	-	-
1 episode	74	11.9 (1.12)	-0.02 (-0.36, 0.31)	0.884
2+ episodes	35	11.8 (1.18)	0.01 (-0.42, 0.44)	0.952

Table 5.10 Incidence of maternal anaemia (Hb <11.0g/dl) by number of episodes of asymptomatic sub-patent RDT parasitaemia

Parasitaemia level	**Number of women**	**Total number of events**	**Total person-years follow-up**	**Incidence rate (/1,000 pyrs)**	**Crude incidence rate ratio (95% C.I)**	**P-value**	**Adjusted incidence rate ratio (95% C.I)**	**P-value**
G1+2							†	
No parasitaemia (reference)	70	49	33.98	1,442.02	-	-	-	-
1 episode	50	35	23.20	1,508.62	1.05 (0.66, 1.65)	0.845	0.92 (0.71, 1.19)	0.508
2+ episodes	20	6	9.25	648.65	0.45 (0.16, 1.23)	0.120	0.63 (0.38, 1.04)	0.072
G3+							†	
No parasitaemia (reference)	85	40	42.01	952.15	-	-	-	-
1 episode	47	29	20.61	1,407.08	1.47 (0.82, 2.66)	0.193	1.13 (0.78, 1.62)	0.517
2+ episodes	24	17	11.25	1,511.11	1.59 (0.79, 3.18)	0.193	2.17 (1.12, 4.21)	0.021
All							‡	
No parasitaemia (reference)	155	89	75.99	1,171.2	-	-	-	-
1 episode	97	64	43.80	1,461.2	1.25 (0.87, 1.80)	0.235	1.00 (0.80, 1.26)	0.982
2+ episodes	44	23	20.50	1,122.0	0.96 (0.54, 1.70)	0.882	1.34 (0.86, 2.08)	0.192

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.11 Risk of any placental malaria by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	64	15	15/64 (23.4)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	62	28	28/62 (45.2)	1.93 (1.14, 3.25)	0.014	1.89 (1.14, 3.15)	0.014
Only asymptomatic RDT Patent parasitaemia	103	64	64/103 (62.1)	2.65 (1.69, 4.24)	< 0.001	2.14 (1.32, 3.48)	0.002
G3+						†	
No parasitaemia (reference)	73	13	13/73 (17.8)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	67	22	22/67 (32.8)	1.84 (1.01, 3.37)	0.046	1.50 (0.82, 2.73)	0.186
Only asymptomatic RDT Patent parasitaemia	39	15	15/39 (38.5)	2.16 (1.14, 4.07)	0.017	1.71 (0.87, 3.33)	0.117
All						‡	
No parasitaemia (reference)	137	28	28/137 (20.4)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	129	50	50/129 (38.8)	1.90 (1.28, 2.82)	0.002	1.77 (1.20, 2.61)	0.004
Only asymptomatic RDT Patent parasitaemia	142	79	79/142 (55.6)	2.72 (1.90, 3.91)	< 0.001	2.00 (1.37, 2.93)	<0.001

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.12 Risk of any placental malaria by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	64	15	15/64 (23.4)	-	-	-	-
Second trimester only	25	7	7/25 (28.0)	1.19 (0.55, 2.58)	0.651	1.22 (0.57, 2.61)	0.601
Third trimester only	30	16	16/30 (53.3)	2.28 (1.30, 3.97)	0.004	2.14 (1.24, 3.69)	0.006
Second and Third trimester	7	5	5/7 (71.4)	3.05 (1.60, 4.24)	0.001	3.04 (1.66, 5.58)	< 0.001
G3+						†	
No parasitaemia (reference)	73	13	13/73 (17.8)	-	-	-	-
Second trimester only	23	3	3/23 (13.0)	0.73 (0.22, 2.35)	0.601	0.64 (0.19, 2.18)	0.473
Third trimester only	29	11	11/29 (37.9)	2.13 (1.08, 4.20)	0.029	1.64 (0.82, 3.27)	0.162
Second and Third trimester	15	8	8/15 (53.3)	2.99 (1.51, 5.94)	0.002	2.69 (1.38, 5.23)	0.004
All						‡	
No parasitaemia (reference)	137	28	28/137 (20.4)	-	-	-	-
Second trimester only	48	10	10/48 (20.8)	1.02 (0.54, 1.94)	0.953	0.97 (0.51, 1.84)	0.924
Third trimester only	59	27	27/59 (45.8)	2.24 (1.45, 3.45)	< 0.001	1.99 (1.31, 3.04)	0.001
Second and Third trimester	22	13	13/22 (59.1)	2.89 (1.79, 4.67)	< 0.001	3.07 (1.93, 4.87)	< 0.001

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.13 Risk of any placental malaria by number of episodes of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	64	15	15/64 (23.4)	-	-	-	-
1 episode	43	16	16/43 (37.2)	1.59 (0.88, 2.86)	0.125	1.51 (0.85, 2.67)	0.160
2+ episodes	19	12	12/19 (63.2)	2.69 (1.54, 4.73)	0.001	2.85 (1.66, 4.90)	< 0.001
G3+						†	
No parasitaemia (reference)	73	13	13/73 (18.8)	-	-	-	-
1 episode	46	13	13/46 (28.3)	1.59 (0.81, 3.12)	0.181	1.22 (0.62, 2.42)	0.565
2+ episodes	21	9	9/21 (42.9)	2.41 (1.20, 4.84)	0.014	2.19 (1.11, 4.31)	0.023
All						‡	
No parasitaemia (reference)	137	28	28/137 (20.4)	-	-	-	-
1 episode	89	29	29/89 (32.6)	1.59 (1.02, 2.49)	0.040	1.42 (0.92, 2.20)	0.118
2+ episodes	40	21	21/40 (52.5)	2.57 (1.65, 4.00)	< 0.001	2.69 (1.76, 4.12)	< 0.001

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.14 Risk of composite adverse livebirth outcome (SGA/PTB/LBW) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	66	12	12/66 (18.2)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	66	18	18/66 (27.3)	1.50 (0.79, 2.87)	0.220	1.57 (0.81, 3.04)	0.178
Only asymptomatic RDT Patent parasitaemia	103	40	40/103 (38.8)	2.14 (1.21, 3.77)	0.009	1.69 (0.94, 3.07)	0.082
G3+						†	
No parasitaemia (reference)	82	7	7/82 (8.5)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	70	18	18/70 (25.7)	3.01 (1.33, 6.80)	0.008	3.59 (1.53, 8.40)	0.003
Only asymptomatic RDT Patent parasitaemia	42	14	14/42 (33.3)	3.90 (1.70, 8.95)	0.001	4.99 (2.11, 11.80)	< 0.001
All						‡	
No parasitaemia (reference)	148	19	19/148 (12.8)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	136	36	36/136 (26.5)	2.06 (1.24, 3.42)	0.005	2.09 (1.25, 3.48)	0.005
Only asymptomatic RDT Patent parasitaemia	145	54	54/145 (37.2)	2.90 (1.81, 4.64)	< 0.001	2.51 (1.52, 4.13)	< 0.001

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.15 Risk of composite adverse livebirth outcome (SGA/PTB/LBW) by number of episodes of sub-patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	66	12	12/66 (18.2)	-	-	-	-
1 episode	48	11	11/48 (22.9)	1.26 (0.61, 2.61)	0.535	1.33 (0.63, 2.78)	0.454
2+ episodes	18	7	7/18 (38.9)	2.14 (0.99, 4.64)	0.054	2.23 (1.02, 4.88)	0.044
G3+						†	
No parasitaemia (reference)	82	7	7/82 (8.5)	-	-	-	-
1 episode	46	14	14/46 (30.4)	3.57 (1.55, 8.21)	0.003	4.55 (1.91, 10.83)	0.001
2+ episodes	24	4	4/24 (16.7)	1.95 (0.62, 6.13)	0.252	2.13 (0.65, 6.93)	0.209
All						‡	
No parasitaemia (reference)	148	19	19/148 (12.8)	-	-	-	-
1 episode	94	25	25/94 (26.6)	2.07 (1.21, 3.55)	0.008	2.10 (1.22, 3.64)	0.008
2+ episodes	42	11	11/42 (26.2)	2.04 (1.05, 3.95)	0.034	2.06 (1.06, 3.98)	0.032

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.16 Risk of composite adverse livebirth outcome (SGA/PTB/LBW) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	66	12	12/66 (18.2)	-	-	-	-
Second trimester only	28	7	7/28 (25.0)	1.38 (0.60, 3.13)	0.448	1.52 (0.67, 3.49)	0.319
Third trimester only	32	8	8/32 (25.0)	1.38 (0.62, 3.03)	0.430	1.38 (0.63, 3.05)	0.420
Second and Third trimester	6	3	3/6 (50.0)	2.75 (1.06, 7.12)	0.037	2.70 (1.10, 6.59)	0.029
G3+						†	
No parasitaemia (reference)	82	7	7/82 (8.5)	-	-	-	-
Second trimester only	23	8	8/23 (34.8)	4.07 (1.65, 10.07)	0.002	4.67 (1.83, 11.88)	0.001
Third trimester only	29	7	7/29 (24.1)	2.83 (1.08, 7.39)	0.034	3.80 (1.39, 10.40)	0.009
Second and Third trimester	18	3	3/18 (16.7)	1.95 (0.56, 6.85)	0.296	2.07 (0.56, 7.69)	0.276
All						‡	
No parasitaemia (reference)	148	19	19/148 (12.8)	-	-	-	-
Second trimester only	51	15	15/51 (29.4)	2.29 (1.26, 4.17)	0.007	2.34 (1.27, 4.29)	0.006
Third trimester only	61	15	15/61 (24.6)	1.92 (1.04, 3.52)	0.036	1.90 (1.02, 3.51)	0.041
Second and Third trimester	24	6	6/24 (25.0)	1.95 (0.87, 4.38)	0.107	2.03 (0.91, 4.53)	0.084

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

By gravidity, there was a significant difference in birthweights amongst paucigravidae in favor of babies born to women who never had malaria infection (2,928.5 vs 2, 802.8g, mean difference 125.7g, 95% C.I. 1.49, 249.88, p-value = 0.047) and a lower but non-significant difference in multigravidae (3,080.0 vs 2,927.2g, mean difference 152.8, 95% C.I. -11.90, 317.60, p-value = 0.069).

5.2.4.4 Preterm Birth

There was an elevated risk of preterm delivery with asymptomatic sub-RDT parasitaemia in both paucigravidae (21.2 vs 4.6%, RR 4.67, 95% C.I. 1.40, 15.50, p-value = 0.012) and multigravidae (17.1 vs 3.7%, RR 4.69, 95% C.I. 1.37, 15.99, p-value 0.014) (Table 5.26). The timing of asymptomatic infection was significantly associated with increased risk in paucigravidae in the second trimester only or infection occurred in the second and third trimester. This association was however only significant in multigravidae if infection occurred in either the second trimester or third trimester only (Table 5.27). Any number of episodes of asymptomatic sub-RDT infection in paucigravidae was significantly associated with an increased risk of preterm delivery but only one and not 2 or more episodes were associated with a raised risk amongst multigravidae (Table 5.28).

On average, babies born to women without any parasitaemia were 0.69 weeks older than those born to women with any asymptomatic sub-RDT parasitaemia (39.0 vs 38.3 weeks, 95% C.I. 0.33, 1.05, p-value < 0.001). This effect, though slightly varied, was constant in each gravidity strata (Table 5.29). Amongst paucigravidae, there was a significant difference in the mean gestational age at delivery if infection occurred in the second and third trimester and with more than 2 episodes. Comparatively, infection in either the second trimester or third trimester only and only 1 episode of infection was associated with significantly earlier fetal age at delivery in multigravidae (Tables 5.30 and 5.31).

Table 5.17 Risk of SGA (corrected birthweight < 10th percentile for gestational age) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	64	8	8/64 (12.5)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	62	4	4/62 (6.5)	0.52 (0.16, 1.63)	0.260	0.56 (0.17, 1.83)	0.342
Only asymptomatic RDT Patent parasitaemia	101	16	16/101 (15.8)	1.27 (0.57, 2.79)	0.557	1.09 (0.47,2.52)	0.835
G3+						†	
No parasitaemia (reference)	75	5	5/75 (6.7)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	66	6	6/66 (9.1)	1.36 (0.43, 4.28)	0.595	1.73 (0.50, 5.95)	0.386
Only asymptomatic RDT Patent parasitaemia	40	5	5/40 (12.5)	1.88 (0.58, 6.11)	0.297	2.59 (0.76, 8.89)	0.130
All						‡	
No parasitaemia (reference)	139	13	13/139 (9.4)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	128	10	10/128 (7.8)	0.84 (0.38, 1.84)	0.655	0.93 (0.41, 2.09)	0.854
Only asymptomatic RDT Patent parasitaemia	141	21	21/141 (14.9)	1.59 (0.83, 3.06)	0.162	1.52 (0.76, 3.06)	0.240

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.18 Risk of SGA (corrected birthweight < 10th percentile for gestational age) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	64	8	8/64 (12.5)	-	-	-	-
Second trimester only	26	0	0/26 (0.0)	2.20e-07 (1.03e-07, 4.67e-07)	0.000	2.05e-07 (9.05e-08, 4.63e-07)	0.000
Third trimester only	30	4	4/30 (13.3)	1.07 (0.35, 3.27)	0.910	1.16 (0.37, 3.69)	0.798
Second and Third trimester	6	0	0/6 (0.0)	2.20e-07 (7.82e-08, 6.16e-07)	0.000	2.08e-07 (6.91e-08, 6.28e-07)	0.000
G3+						†	
No parasitaemia (reference)	75	5	5/75 (6.7)	-	-	-	-
Second trimester only	23	2	2/23 (8.7)	1.30 (0.27, 6.31)	0.741	1.45 (0.31, 6.82)	0.638
Third trimester only	28	2	2/28 (7.1)	1.07 (0.22, 5.23)	0.932	1.50 (0.30, 7.58)	0.625
Second and Third trimester	15	2	2/15 (13.3)	2.00 (0.43, 9.40)	0.380	2.71 (0.45, 16.1)	0.274
All						‡	
No parasitaemia (reference)	139	13	13/139 (9.4)	-	-	-	-
Second trimester only	49	2	2/49 (4.1)	0.44 (0.10, 1.87)	0.264	0.46 (0.11, 2.01)	0.302
Third trimester only	58	6	6/58 (10.3)	1.11 (0.44, 2.77)	0.830	1.24 (0.50, 3.09)	0.646
Second and Third trimester	21	2	2/21 (9.5)	1.02 (0.25, 4.20)	0.980	1.28 (0.30, 5.43)	0.738

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.19 Risk of SGA (corrected birthweight < 10th percentile for gestational age) by number of episodes of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2							
						†	
No parasitaemia (reference)	64	8	8/64 (12.5)	-	-	-	-
1 episode	44	2	2/44 (4.6)	0.36 (0.81, 1.64)	0.188	0.39 (0.84, 1.76)	0.218
2+ episodes	18	2	2/18 (11.1)	0.89 (0.21, 3.83)	0.875	1.06 (0.25, 4.53)	0.934
G3+							
						†	
No parasitaemia (reference)	75	5	5/75 (6.7)	-	-	-	-
1 episode	45	4	4/45 (8.9)	1.33 (0.38, 4.73)	0.656	1.62 (0.46, 5.71)	0.449
2+ episodes	21	2	2/21 (9.5)	1.43 (0.30, 6.87)	0.656	1.98 (0.34, 11.47)	0.447
All							
						‡	
No parasitaemia (reference)	139	13	13/139 (9.4)	-	-	-	-
1 episode	89	6	6/89 (6.7)	0.72 (0.28, 1.83)	0.491	0.77 (0.31, 1.94)	0.581
2+ episodes	39	4	4/39 (10.3)	1.10 (0.38, 3.18)	0.865	1.33 (0.44, 4.03)	0.610

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.20 Risk of Low birth weight (corrected weight at deliver < 2.50 kg) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	64	6	6/64 (9.4)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	62	4	4/62 (6.5)	0.69 (0.20, 2.33)	0.548	0.76 (0.23, 2.51)	0.654
Only asymptomatic RDT Patent parasitaemia	101	22	22/101 (21.8)	2.32 (0.99, 5.43)	0.051	1.68 (0.71, 3.98)	0.236
G3+						†	
No parasitaemia (reference)	75	3	3/75 (4.0)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	66	5	5/66 (7.6)	1.89 (0.47, 7.65)	0.370	2.41 (0.49, 11.81)	0.278
Only asymptomatic RDT Patent parasitaemia	40	5	5/40 (12.5)	3.13 (0.78, 12.46)	0.106	4.48 (1.24, 16.24)	0.022
All						‡	
No parasitaemia (reference)	139	9	9/139 (6.5)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	128	9	9/128 (7.0)	1.09 (0.44, 2.65)	0.856	1.18 (0.47, 2.92)	0.725
Only asymptomatic RDT Patent parasitaemia	141	27	27/141 (19.1)	2.96 (1.44, 6.06)	0.003	2.39 (1.12, 5.10)	0.024

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.21 Risk of Low birth weight (corrected weight at deliver < 2.50 kg) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	64	6	6/64 (9.4)	-	-	-	-
Second trimester only	26	1	1/26 (3.9)	0.41 (0.05, 3.26)	0.399	0.46 (0.06, 3.70)	0.469
Third trimester only	30	3	3/10 (10.0)	1.07 (0.29, 3.99)	0.924	1.14 (0.33, 3.99)	0.837
Second and Third trimester	6	0	0/6 (0.0)	2.18e-06 (7.21e-07, 6.60e-06)	0.000	8.02e-07 (2.43e-07, 2.65e-06)	0.000
G3+						†	
No parasitaemia (reference)	72	3	3/71 (4.0)	-	-	-	-
Second trimester only	23	2	2/23 (8.7)	2.17 (0.38, 12.29)	0.380	2.69 (0.43, 16.82)	0.291
Third trimester only	28	2	2/28 (7.1)	1.79 (0.31, 10.18)	0.514	2.39 (0.32, 17.68)	0.394
Second and Third trimester	15	1	1/15 (6.7)	1.67 (0.18, 15.05)	0.649	1.99 (0.17, 23.32)	0.585
All						‡	
No parasitaemia (reference)	139	9	9/139 (6.5)	-	-	-	-
Second trimester only	49	3	3/49 (6.1)	0.95 (0.27, 3.36)	0.931	1.00 (0.27, 3.66)	0.994
Third trimester only	58	5	5/58 (8.6)	1.33 (0.47, 3.81)	0.593	1.44 (0.50, 4.13)	0.493
Second and Third trimester	21	1	1/21 (4.8)	0.74 (0.10, 5.53)	0.765	0.86 (0.11, 6.51)	0.886

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.22 Risk of Low birth weight (corrected weight at deliver < 2.50 kg) by number of episodes of sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	64	6	6/64 (9.4)	-	-	-	-
1 episode	44	2	2/44 (4.6)	0.48 (0.10, 2.30)	0.362	0.50 (0.11, 2.25)	0.370
2+ episodes	18	2	2/18 (11.1)	1.19 (0.26, 5.40)	0.836	1.57 (0.35, 7.02)	0.557
G3+S						†	
No parasitaemia (reference)	75	3	3/75 (4.0)	-	-	-	-
1 episode	45	3	3/45 (6.7)	1.67 (0.35, 7.94)	0.521	2.15 (0.36, 12.73)	0.398
2+ episodes	21	2	2/21 (9.5)	2.38 (0.42, 13.39)	0.325	2.94 (0.4917.56)	0.236
All						‡	
No parasitaemia (reference)	139	9	9/139 (6.5)	-	-	-	-
1 episode	89	5	5/89 (5.6)	0.87 (0.30, 2.51)	0.793	0.92 (0.31, 2.70)	0.882
2+ episodes	39	4	4/39 (10.3)	1.58 (0.51, 4.88)	0.423	1.81 (0.58, 5.71)	0.309

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.23 Difference in mean birthweight by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean corrected birthweight g** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	64	2,928.5 (348.40)	-	-
Only asymptomatic sub-RDT parasitaemia	62	2,922.1 (367.64)	6.37 (-119.89, 132.64)	0.921
Only asymptomatic RDT Patent parasitaemia	101	2,802.8 (419.69)	125.69 (1.49, 249.88)	0.047
G3+				
No parasitaemia (reference)	75	3,080.0 (405.65)	-	-
Only asymptomatic sub-RDT parasitaemia	66	3,104.6 (449.38)	-24.61 (-166.98, 117.77)	0.733
Only asymptomatic RDT Patent parasitaemia	40	2,927.15 (458.78)	152.85 (-11.90, 317.60)	0.069
All				
No parasitaemia (reference)	139	3,010.25 (386.52)	-	-
Only asymptomatic sub-RDT parasitaemia	128	3,016.33 (420.31)	-5.97 (-103.19, 91.25)	0.904
Only asymptomatic RDT Patent parasitaemia	141	2,838.09 (433.14)	172.16 (75.54, 268.78)	< 0.001

Table 5.24 Difference in mean birthweight by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean corrected birthweight** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	64	2,928.5 (348.40)	-	-
Second trimester only	26	2,983.2 (379.87)	-54.7 (-219.94, 110.63)	0.513
Third trimester only	30	2,867.0 (363.67)	61.5 (-93.75, 216.75)	0.433
Second and Third trimester	6	2,933.3 (350.23)	-4.8 (-301.78, 292.11)	0.974
G3+				
No parasitaemia (reference)	75	3,080.0 (405.65)	-	-
Second trimester only	23	3,116.1 (498.11)	-36.1 (-238.87, 166.70)	0.725
Third trimester only	28	3,138.3 (454.54)	-58.3 (-242.49, 125.92)	0.532
Second and Third trimester	15	3,024.1 (373.93)	55.9 (-169.40, 281.14)	0.623
All				
No parasitaemia (reference)	139	3,010.2 (386.52)	-	-
Second trimester only	49	3,045.6 (439.74)	-35.3 (-166.71, 96.10)	0.597
Third trimester only	58	2,998.0 (428.78)	12.3 (-110.83, 135.39)	0.844
Second and Third trimester	21	2,998.2 (360.98)	12.1 (-165.22, 189.33)	0.893

Table 5.25 Difference in mean birthweight by frequency of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean corrected birthweight** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	64	2,928.5 (348.40)	-	-
1 episode	44	2,944.8 (374.38)	-16.3 (-155.77, 123.13)	0.817
2+ episodes	18	2,866.7 (354.80)	61.8 (-123.87, 247.54)	0.510
G3+				
No parasitaemia (reference)	75	3,080.0 (405.65)	-	-
1 episode	45	3,125.4 (453.90)	-45.4 (-203.66, 112.90)	0.571
2+ episodes	21	3,060.1 (449.47)	19.9 (-183.70, 223.51)	0.847
All				
No parasitaemia (reference)	139	3,010.2 (386.52)	-	-
1 episode	89	3,036.1 (423.42)	-25.9 (-133.22, 81.48)	0.635
2+ episodes	39	2,970.8 (414.95)	39.4 (-101.06, 179.91)	0.580

Table 5.26 Risk of Preterm Birth (corrected gestational age at birth < 37 completed weeks) by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Number of women with event (n)	Risk n/N (%)	Crude risk ratio (95% C.I)	P-value	Adjusted risk ratio (95% C.I)	P-value
G1+2						†	
No parasitaemia (reference)	66	3	3/66 (4.6)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	66	14	14/66 (21.2)	4.67 (1.40, 15.50)	0.012	4.84 (1.46, 16.10)	0.010
Only asymptomatic RDT Patent parasitaemia	103	27	27/103 (26.2)	5.77 (1.82, 18.30)	0.003	4.08 (1.22, 13.66)	0.022
G3+						†	
No parasitaemia (reference)	82	3	3/82 (3.7)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	70	12	12/70 (17.1)	4.69 (1.37, 15.99)	0.014	5.41 (1.61, 18.22)	0.006
Only asymptomatic RDT Patent parasitaemia	42	13	13/42 (31.0)	8.46 (2.54, 28.15)	<0.001	11.31 (3.35, 38.23)	< 0.001
All						‡	
No parasitaemia (reference)	148	6	6/148 (4.1)	-	-	-	-
Only asymptomatic sub-RDT parasitaemia	136	26	26/136 (19.1)	4.72 (2.00, 11.12)	< 0.001	4.57 (1.94, 10.73)	< 0.001
Only asymptomatic RDT Patent parasitaemia	145	40	40/145 (27.6)	6.80 (2.97, 15.57)	< 0.001	6.01 (2.52, 14.30)	< 0.001

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.27 Risk of Preterm Birth (corrected gestational age at birth < 37 completed weeks) by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	66	3	3/66 (4.6)	-	-	-	-
Second trimester only	28	6	6/28 (21.4)	4.71 (1.26, 17.58)	0.021	5.36 (1.44, 20.00)	0.012
Third trimester only	32	5	5/32 (15.6)	3.44 (0.87, 13.54)	0.077	3.37 (0.88, 12.97)	0.077
Second and Third trimester	6	3	3/6 (50.0)	11.00 (2.80, 43.19)	0.001	9.54 (2.61, 34.92)	0.001
G3+						†	
No parasitaemia (reference)	82	3	3/82 (3.7)	-	-	-	-
Second trimester only	23	6	6/23 (26.1)	7.13 (1.92, 26.42)	0.003	8.74 (2.44, 31.28)	0.001
Third trimester only	29	5	5/29 (17.2)	4.71 (1.20, 18.56)	0.027	6.47 (1.69, 24.82)	0.006
Second and Third trimester	18	1	1/18 (5.6)	1.52 (0.17, 13.85)	0.711	1.38 (0.15, 12.64)	0.773
All						‡	
No parasitaemia (reference)	148	6	6/148 (4.1)	-	-	-	-
Second trimester only	51	12	12/51 (23.5)	5.80 (2.29, 14.68)	< 0.0001	6.03 (2.41, 15.10)	< 0.0001
Third trimester only	61	10	10/61 (16.4)	4.04 (1.54, 10.65)	0.005	3.79 (1.44, 9.98)	0.007
Second and Third trimester	24	4	4/24 (16.7)	4.11 (1.25, 13.52)	0.020	3.69 (1.12, 12.14)	0.032

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.28 Risk of Preterm Birth (corrected gestational age at birth < 37 completed weeks) by number of episodes of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Number of women with event (n)**	**Risk n/N (%)**	**Crude risk ratio (95% C.I)**	**P-value**	**Adjusted risk ratio (95% C.I)**	**P-value**
G1+2						†	
No parasitaemia (reference)	66	3	3/66 (4.6)	-	-	-	-
1 episode	48	8	8/48 (16.7)	3.67 (1.02, 13.14)	0.046	3.82 (1.06, 13.79)	0.041
2+ episodes	18	6	6/18 (33.3)	7.33 (2.02, 26.56)	0.002	7.61 (2.19, 26.41)	0.001
G3+						†	
No parasitaemia (reference)	82	3	3/82 (3.7)	-	-	-	-
1 episode	46	10	10/46 (21.7)	5.94 (1.72, 20.57)	0.005	8.06 (2.43, 26.67)	0.001
2+ episodes	25	2	2/25 (8.3)	2.28 (0.40, 12.91)	0.352	2.17 (0.39, 11.98)	0.372
All						‡	
No parasitaemia (reference)	148	6	6/148 (4.1)	-	-	-	-
1 episode	94	18	18/94 (19.1)	4.72 (1.94, 11.48)	0.001	4.75 (1.95, 11.55)	0.001
2+ episodes	42	8	8/42 (19.0)	4.70 (1.72, 12.81)	0.002	4.22 (1.56, 11.39)	0.005

† adjusted for all baseline characteristics excluding gravidity
‡ adjusted for all baseline characteristics including gravidity

Table 5.29 Difference in mean gestational age at delivery by asymptomatic sub-patent RDT and asymptomatic patent RDT parasitaemia

Parasitaemia	Number of women (N)	Mean gestational age at delivery (sd)	Mean difference (95% C.I)	P-value
G1+2				
No parasitaemia (reference)	67	38.9 (1.20)	-	-
Only asymptomatic sub-RDT parasitaemia	67	38.3 (1.76)	0.60 (0.09,1.12)	0.022
Only asymptomatic RDT Patent parasitaemia	106	37.7 (2.35)	1.18 (0.57, 1.79)	< 0.001
G3+				
No parasitaemia (reference)	84	39.0 (1.34)	-	-
Only asymptomatic sub-RDT parasitaemia	71	38.2 (1.89)	0.76 (0.25, 1.28)	0.004
Only asymptomatic RDT Patent parasitaemia	42	37.8 (2.14)	1.22 (0.60, 1.83)	< 0.001
All				
No parasitaemia (reference)	151	39.0 (1.28)	-	-
Only asymptomatic sub-RDT parasitaemia	138	38.3 (1.82)	0.69 (0.33, 1.05)	< 0.001
Only asymptomatic RDT Patent parasitaemia	148	37.8 (2.28)	1.21 (0.79, 1.63)	< 0.001

Table 5.30 Difference in mean gestational age at delivery by timing of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean gestational age at delivery** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	67	38.9 (1.20)	-	-
Second trimester only	28	38.4 (1.81)	0.57 (-0.05, 1.20)	0.073
Third trimester only	32	38.5 (1.58)	0.43 (-0.14, 1.00)	0.134
Second and Third trimester	7	37.4 (2.28)	1.50 (0.45, 2.55)	0.006
G3+				
No parasitaemia (reference)	84	39.0 (1.34)	-	-
Second trimester only	23	38.3 (1.72)	0.70 (0.04, 1.37)	0.039
Third trimester only	30	38.0 (2.32)	1.02 (0.33, 1.72)	0.004
Second and Third trimester	18	38.6 (1.18)	0.41 (-0.26, 1.09)	0.230
All				
No parasitaemia (reference)	151	39.0 (1.28)	-	-
Second trimester only	51	38.3 (1.75)	0.63 (0.19, 1.09)	0.006
Third trimester only	62	38.2 (1.97)	0.72 (0.27, 1.17)	0.002
Second and Third trimester	25	38.3 (1.60)	0.71 (0.14, 1.27)	0.015

Table 5.31 Difference in mean gestational age at delivery by frequency of asymptomatic sub-patent RDT parasitaemia

Parasitaemia	**Number of women (N)**	**Mean gestational age at delivery** (sd)	**Mean difference (95% C.I)**	**P-value**
G1+2				
No parasitaemia (reference)	67	38.9 (1.20)	-	-
1 episode	48	38.5 (1.62)	0.45 (-0.72, 0.97)	0.091
2+ episodes	19	37.9 (2.05)	0.99 (0.26, 1.73)	0.009
G3+				
No parasitaemia (reference)	84	39.0 (1.34)	-	-
1 episode	47	38.1 (2.12)	0.92 (0.32, 1.51)	0.003
2+ episodes	24	38.5 (1.32)	0.47 (-0.15, 1.08)	0.135
All				
No parasitaemia (reference)	151	39.0 (1.28)	-	-
1 episode	95	38.3 (1.88)	0.68 (0.29, 1.08)	< 0.001
2+ episodes	43	38.3 (1.68)	0.70 (0.23, 1.17)	0.004

There was strong evidence of a raised risk of preterm delivery with asymptomatic patent RDT parasitaemia (27.6 vs 4.1%, RR 6.80, 95% C.I. 2.97, 15.57, p-value < 0.001). Babies born to mothers who never had parasitaemia were more than a week older on average at delivery compared to those born to mothers with at least one episode of asymptomatic patent infection (39.0 vs 37.8 weeks, mean difference 1.21, 95% C.I. 0.79, 1.63 weeks, p-value < 0.001).

5.2.5 Discussion

In our analysis, we found that asymptomatic RDT parasitaemia had different effects on pregnancy outcomes in both the mother and foetus depending on its characterization by RDT detection. Asymptomatic sub-RDT infection only was associated with an increased risk of any adverse live birth outcome, largely as a result of the significantly higher risk of preterm birth in women with such infections. In addition, asymptomatic sub-RDT infection was associated with a higher risks of any placental malaria, and this was observed in both pauci- and multigravidae.

The risk of preterm birth and placental malaria was elevated with asymptomatic infection in the second or third trimester. A frequency of two or more episodes of infection were significantly associated with higher risks for preterm birth and placental malaria in paucigravidae whilst these were significantly increased in multigravidae with only one episode of asymptomatic sub-RDT infection.

Asymptomatic but patent RDT infections detected during at least one antenatal visit was associated with higher risk for maternal anaemia, placental malaria, low birth weight and preterm delivery. Pregnancies exposed to patent RDT infection also had lower haemoglobin at the final scheduled antenatal visit, lower mean birthweights and earlier deliveries. There was however no significant association with small for gestational age by either type of asymptomatic infection.

The results from this study showed a clear trend in a reduction in the prevalence of RDT patent malaria infection with subsequent antenatal visits especially when compared to the initial

booking visit. This may be due to the effect of screening and treating out RDT-positive infection women, most of which were detected during the first visit, followed by further treatment at each subsequent visit, such that fewer women would be parasiteamic by the next visit.

Treating out RDT infection may not be the sole reason for the observed decline in patent RDT infections. Early publications have demonstrated a similar trend in reduction in patent malaria prevalence in settings with alternative chemoprevention strategies (357). In a weekly prospective and initial chemoprevention naïve pregnancy cohort study in western Kenya, the prevalence of microscopically detectable malaria infection was highest at first antenatal visit between 91 and 112 days gestation then tended to taper to lower levels by delivery. This was postulated to be as a result of subsequent higher recovery rates from previous infections, markedly after 98 days gestation, than the occurrence of new infections. It was stated that this came about as a result of "women losing their age dependent immunity at the beginning of pregnancy then to reacquire it within the space of 9 months". This phenomenon has been attributed in recent studies from Benin and Cameroon, to the development of VAR2CSA antibody expression as a function of gestational age (358, 359).

A second explanation for the reduction in patent RDT infection may lie in the distribution of bed nets at the enrolment visit. A previous study conducted in western Kenya showed ITNs reduced the incidence of malaria parasitaemia by 38% over the course of pregnancy (360). Furthermore, a systematic review of the effect of insecticide treated bed nets demonstrated that in two trials, there was a reduction in the time to first parasitaemia, clinical malaria and geometric mean parasite densities among paucigravidae protected by ITNs (361).

The sensitivity of malaria RDTs to detect parasitaemia in the peripheral blood is largely influenced by parasite density and improves with higher parasite densities especially above 200 parasites /ul ((362-364). The trend in reduction of the sensitivity in the RDT to detect peripheral parasitaemia may also be as a result of the evolving maternal immune system over the course of same pregnancy, limiting the number and density of infections probably due to an amelioration of cytokine

and hormonal mechanisms initially mounted to prevent the rejection of the fetal allograft in early pregnancy. A marked increase in corticosteroids, that impede cell mediated malaria immunity, in the third trimester (244) may explain the rise in parasitaemia evident at delivery. Consequently this means that RDTs are more useful early in pregnancy at the first antenatal visit, with less utility in the period in-between. This limitation of only being able to reliably diagnose malaria parasitaemia at higher parasite densities at a single point early in pregnancy poses a significant disadvantage to pre-emptively diagnosing and treating malaria parasitaemia at levels before pathophysiological processes that culminate in adverse pregnancy events are set in motion during the overall course of pregnancy.

In a cross sectional study, asymptomatic malaria infections were demonstrated to be associated with maternal anaemia (365). Our findings have been consistent with these previous results but have further demonstrated that asymptomatic infections not detected by RDT are not associated with maternal anaemia possibly owing to the low density of such infections. Conversely, it was asymptomatic patent RDT infections that were associated with maternal anaemia and this may be likely due to higher parasite densities which have been shown to be strongly correlated with maternal anaemia (366) over the course of pregnancy possibly due to an induction of increased splenic destruction of infected red blood cells in a dose dependent manner.

High parasite densities have been associated with decreased birthweight and increased risk of low birthweight (367, 368) as a result of either reduced availability of nutrients to the foetus due to nutrient depletion during pathophysiological processes of localized infection or mechanical blockage via placental membrane thickening following inflammatory reactions (96). The results from our analysis did not find any association of asymptomatic sub-RDT parasitaemia with low birthweight or mean birthweight. This may suggests that the pathophysiological processes leading to low birthweight are dependent on higher parasite densities as asymptomatic infections detected by RDT had an increased risk for low birthweight and significant difference in birthweight, which

may be enacted in a manner dependant on the timing and frequency of such infections (369). Some studies have reported an increased risk of low birthweight with malaria infection in the first trimester (370, 371). This aspect of timing of infection was not evaluated in the current analysis as women were recruited in the second trimester. It may be possible that sub-RDT infections in the first trimester and chronically carried through pregnancy could result in low birthweight from intrauterine growth restriction following a prolonged carriage of low lying infection. However, neither form of asymptomatic infection was associated with small for gestational age which is rather surprising. This may indicate that asymptomatic malaria infection may not play a directly significant role in intrauterine growth restriction but may be facilitated through other placental microbiotas, such as bacteria, that are supported in the presence of malaria infection. Previous evidence has demonstrated that malaria infection is a significant risk factor for invasive non-typhoid salmonella infection in children and these infections commonly occur as coinfections (372-374).

Acute high density malaria infections, often associated with constitutional symptoms, have been shown to increase the risk of preterm delivery (375) especially in low transmission settings. The risk for preterm delivery in high transmission settings, such as from the where the cohort of this analysis were drawn, is associated with chronic malaria infection (376). The exact mechanism is not clear but may be associated with gradual increase in cytokines due to the persistence of parasitaemia that remains untreated that precipitate the labor cascade (377). The findings in our analysis demonstrate that both asymptomatic sub-RDT parasitaemia and patent RDT infection were associated with preterm delivery. The effect of timing and frequency of asymptomatic sub-RDT parasitaemia was strikingly similar in associations with preterm birth and any placental malaria when examined by gravidity.

Our results are consistent with findings from recently published results from cross-sectional studies from the DRC and Burkina Faso that found that asymptomatic RDT infections increased the odds of maternal anaemia (365, 378). However, given the prospective nature of our study population,

this association was true for women having at least one episode of asymptomatic patent RDT infection. Our data may additionally suggest that by the time of diagnosis of levels of parasitaemia associated with anaemia, this may be too late as the damage would have already been done. This is further supported by the findings that sub-RDT infection was not associated with maternal anaemia, neither by timing nor by frequency.

In the midst of the growing interest in intermittent screening and treatment for the control of malaria in pregnancy as an alternative strategy to intermittent presumptive treatment with SP in current areas of malaria endemicity where SP is failing or have no currently recommended drug strategy, very few studies have evaluated the impact of asymptomatic infections on pregnancy outcomes to warrant the importance of screening for asymptomatic infection and more so, the consequences of missed infections. Our findings indicate that asymptomatic infections have a significant impact on pregnancy outcomes. Low density infections that are missed by RDT have adverse consequences on fetal outcomes whilst patent infections, representing higher degrees of parasitaemia bear significant consequences on maternal and fetal outcomes which may indicate a delay in addressing clinically significant parasitaemia by the time of diagnosis. These findings therefore indicate that if screening were to be incorporated as part of a viable strategy for the control of malaria in pregnancy in order to prevent adverse pregnancy outcomes, the screening test should be able to detect low levels of parasitaemia before reaching asymptomatic patent levels where it would be too late to intervene. Where asymptomatic sub-RDT infection is associated with adverse pregnancy outcomes such as with preterm delivery, a screening strategy would not be ideal and a presumptive treatment strategy with an efficacious agent would be more favourable.

6 CHAPTER SIX: THE PERFORMANCE OF HISTIDINE RICH PROTEIN 2 (HRP2)/ PLASMODIUM LACTASE DEHYDROGENASE (PLDH) MALARIA RAPID DIAGNOSTIC TEST TO DETECT ACTIVE PLACENTAL MALARIA; IMPLICATIONS FOR THE EFFICACY OF INTERMITTENT SCREENING AND TREATMENT FOR THE CONTROL OF MALARIA IN PREGNANCY WITH PLACENTAL SEQUESTRATION

6.1 Statistical considerations and analyses

6.1.1 Rationale

P.falciparum is the only currently known species of malaria to sequester in the placenta with characteristic morphological features on histological examination. Because of this phenomenon, peripheral parasiteamia underestimates the overall density of infection in pregnant women due to the sequestration of a potentially significant biomass in the placenta (379-382). Because of this density mismatch presented by peripheral parasiteamia and the density dependant reliability of RDT performance, intermittent screening and treatment may miss treating a significant mass of parasiteamia that would lead to adverse pregnancy outcomes.

6.1.2 Approach

The study was imbedded in the larger clinical trial comparing the efficacy and safety of intermittent screening and treatment to intermittent presumptive treatment in pregnancy with Sulphadoxine – Pyrimethamine for the control of malaria in pregnancy. The trial and its methods have been described in chapter 3 including laboratory methods for light microscopy, histology, RDT and PCR evaluations. The power of this analysis was thus based on the number of deliveries from the parent trial for which RDT (First Response® Combo Malaria Ag (pLDH/HRP2); Premier Medical Corporation, Ltd, India) results at delivery and placental histology were available. Thus a separate sample size calculation was not conducted for this sub-analysis. Where applicable, guidelines in accordance to the Standard of Reporting of Diagnostic Accuracy (STARD) statement were followed to report results.

6.1.3 Analyses

Stata version 13 (StataCorp, College Station, Texas) with the user written programme stb59 sbe36_1 (http://www.stata.com/stb/stb59) was used to calculate the sensitivity, specificity, positive

predictive value and negative predictive value, and their 95% confidence intervals. The positive and negative likelihood ratios, and diagnostic odds ratio (DOR) were calculated from a Microsoft Excel™ 2013 (Microsoft Corporation, Redmond, Washington) online confidence interval calculator (http://www.pedro.org.au/english/downloads/confidence-interval-calculator/). The DOR is a measure of the effectiveness of a diagnostic test given by the ratio of the odds of a true positive result to the odds of a false positive. Placental histology was considered the gold standard. The sensitivities of diagnostics tests within and between samples were compared using McNemar's test for correlated proportions (383-385) . Statistical significance was set at α of 0.05.

6.2 Results

6.2.1 Patient disposition

Of the 1,873 women enrolled in the trial 1,261 (67.3%) were included in the analysis, having complete data at delivery on peripheral malaria RDT, light microscopy and PCR, and placental malaria detected by RDT, LM, PCR, placental histology and placental impression smears (Figure 6.2). The mean age (SD) was 22.6 (5.0) years and 60.6% of the women were paucigravidae. The majority of women (99.4%) resided in a rural setting. All the women reported having used an insecticide treated net during the current pregnancy with 99.5% having slept under an insecticide treated net 24 hours prior to delivery. Less than 1% of the women reported having felt ill prior to delivery since the last study visit with 0.32% diagnosed with clinical malaria at delivery. The prevalence of peripheral parasitaemia was 8.6%, 2.3% and 20.6% by RDT, LM and PCR respectively. 26.7% of the women had evidence of placental infection, 11.6% of which was active infection. By light microscopy, *P.falciparum* accounted for the vast majority of infections (93.1%) with P.*malariae* contributing the remainder of infections. Mixed infections were not detected.

6.2.2 Performance of peripheral blood malaria rapid diagnostic test for detection of active placental malaria (acute or chronic)

The sensitivity of peripheral blood RDT was 48.0%, with specificity, PPV, NPV, LR+, LR- and DOR values of 96.5%, 64.2%, 93.4%, 13.7, 0.54 and 25.4 respectively (Table 6.1). Comparative performance measures for peripheral blood LM and PCR were higher compared to RDT. PCR was more sensitive than RDT (64.4 vs 47.9%, difference 16.4%, 95% C.I. 8.40, 24.5%, $p < 0.001$) and LM (64.4 vs 16.4%, difference 47.9%, 95% C.I. 38.9, 57.0%, $p < 0.001$). RDT was more sensitive than LM (48.0 vs 16.4%; difference 31.5%, 95% C.I. 22.4, 40.6%, $p < 0.001$).

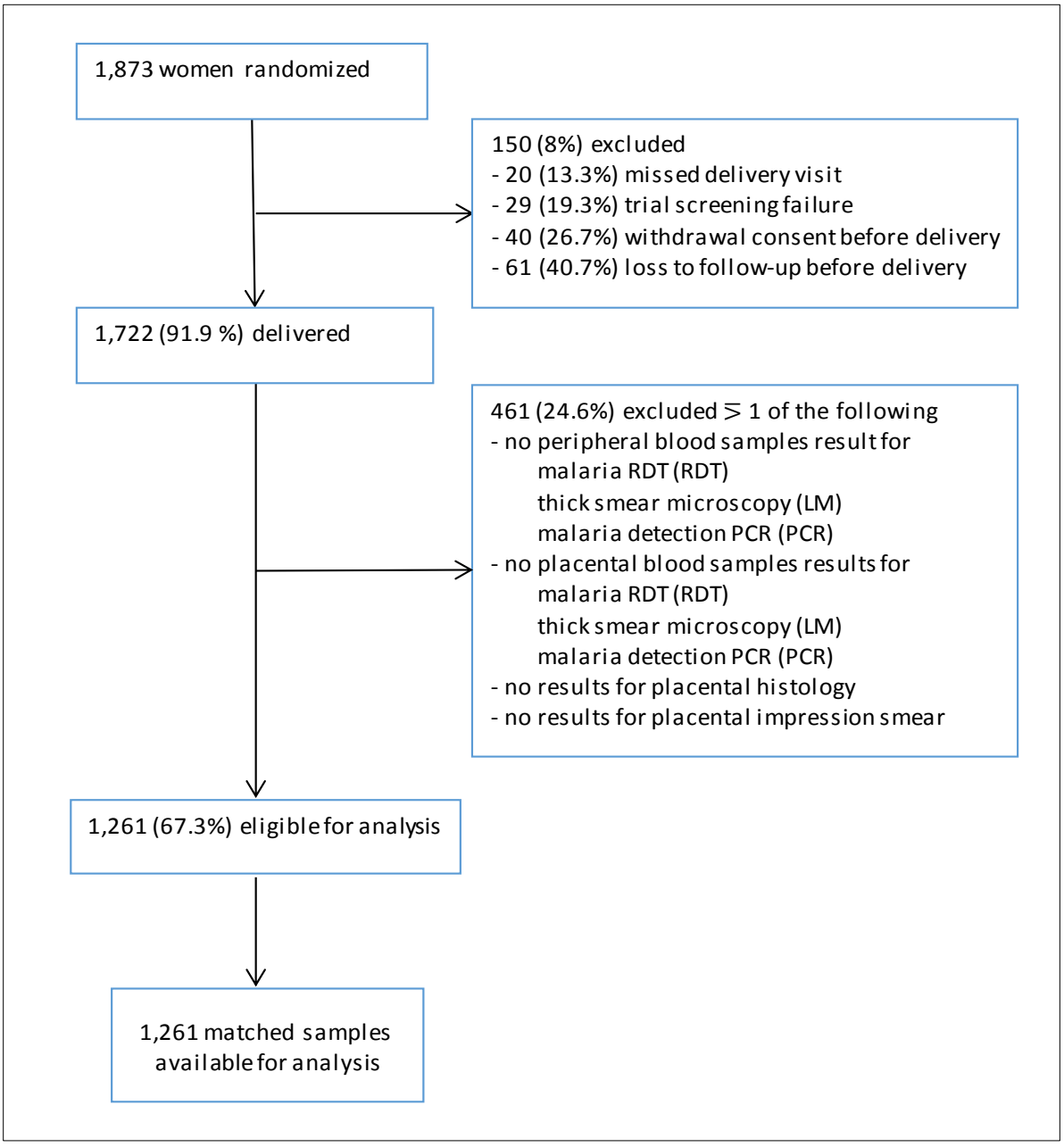
6.2.3 Performance of RDT, LM and PCR in placental blood to detect active placental malaria detected by histology

From placental blood samples, the sensitivity, specificity, PPV, NPV, LR+, LR- and DOR for RDT was 34.3%, 98.8%, 79.4%, 92.0%, 29.4, 0.67 and 44.2 respectively (Table 6.2). LM and PCR evaluation of placental blood to detect active placental malaria were further compared to RDT. As with peripheral blood, PCR remained more sensitive than RDT (67.8 vs 34.2%, difference 33.6%, 95% C.I. 24.3, 42.8%, $p < 0.001$) and LM (67.8 vs 5.5%; difference 62.3%, 95% C.I. 53.4, 71.3%, $p < 0.001$). RDT was significantly more sensitive than LM (34.2 vs 5.5%, difference 28.8%, 95% C.I. 20.5, 37.0%, $p < 0.001$).

6.2.4 Performance of diagnostic tests between peripheral and placental blood samples

The sensitivity for PCR to detect active placental infections was similar between placental and peripheral blood samples (67.8 vs 64.4%, difference 3.42%, 95% C.I. -4.7, 11.2%, $p = 0.369$). However, for both RDT and LM, sensitivity was lower in the placental sample than the peripheral blood sample (34.3 vs 48.0 %: difference -13.7%, 95% C.I. -21.1, -6.3%, $p < 0.001$) and (5.5 vs 16.4%, difference 11.0%, 95% C.I. -17.4, -4.5%, $p < 0.001$).

Figure 6.1 Participant flow chart for diagnostic accuracy eligibility



6.2.5 Association of placental parasite density and the risk of low birth weight

Overall, placental parasitaemia was associated with LBW (Chi 2, P = 0.007), with an almost 1.5 times increased risk (crude RR 1.47, 95% C.I. 1.16, 1.86, p = 0.001) than having no placental parasitaemia. The risk ratio for LBW increased with placental parasite density, with a statistically significant effect above 100 parasites/500RBC (crude RR 3.98, 95% C.I 1.78, 8.94, p = 0.001). This was a mainly pronounced effect amongst paucigravidae with risk in multigravidae not being significant (Table 6.3).

6.2.6 Peripheral malaria rapid diagnostic test performance by placental parasite density

The influence of placental parasite density on the sensitivity of peripheral RDT to diagnose active placental malaria showed an increase in sensitivity with an increase in the placental parasite density though this increase was minimal above 50parasites/500RBC (Table 6.4). Comparatively, at a density of >100parasites / 500 RBC, PCR demonstrated a sensitivity of 91.3%, 95% C.I. 72.0, 98.9%.

Table 6.1 Summary performance measures of diagnostic tests for peripheral parasitaemia to detect active placental malaria by placental histology

Index test	**Performance parameter (95% C.I)**						
	Sensitivity	Specificity	PPV	NPV	LR+	LR-	DOR
RDT	48.0 (39.6 – 56.4)	96.5 (95.3 – 97.5)	64.2 (54.5 – 73.2)	93.4 (91.8 – 94.8)	13.7 (9.6 – 19.5)	0.54 (0.56 – 0.63)	25.4 (16.1 – 40.1)
LM	16.4 (10.8 - 23.5)	99.6 (99.0 - 99.9)	82.8 (64.2 - 94.2)	90.1 (88.3 - 91.7)	36.7(14.2 – 94.6)	0.83 (0.78 – 0.90)	43.7 (16.4 – 116.5)
PCR	64.4 (56.0 - 72.1)	85.1 (82.9 - 87.2)	36.1 (30.3 - 42.3)	94.8 (93.2 - 96.1)	4.3 (3.6 – 5.2)	0.42 (0.34 – 0.52)	10.3 (7.09 – 15.1)

Table 6.2 Summary performance measures of diagnostic tests for peripheral parasitaemia to detect active placental malaria

Index test	**Performance parameter (95% C.I)**						
	Sensitivity	Specificity	PPV	NPV	LR+	LR-	DOR
RDT	34.3 (26.6 – 42.6)	98.8 (98.0 – 99.4)	79.4 (67.3 – 88.5)	92.0 (90.3 – 93.5)	29.4 (16.4 – 52.7)	0.67 (0.59 – 0.75)	44.2 (23.2 – 84.1)
LM	5.5 (2.4 - 10.5)	99.6 (99.1 - 99.9)	66.7 (34.9 - 90.1)	89.0 (87.1 - 90.6)	15.2 (4.7 – 50.1)	0.95 (0.91 – 0.99)	16.1 (4.8 – 54.2)
PCR	67.8 (59.6 - 75.3)	87.7(85.6 - 89.6)	42.0 (35.6 - 48.3)	95.4 (94.0 - 96.6)	5.5 (4.6 – 6.7)	0.37 (0.29 – 0.47)	15.0 (10.2 – 22.2)

Table 6.3 Association of placental parasite density and low birthweight, stratified by gravidity

Placental parasite density (n/ 500 RBCs)	Total Number of women	Number of women with LBW outcome	Risk	Crude Risk Ratio (95% C.I)	P-value	Adjusted Risk Ratio (95% C.I)†	P-value
G1+2							
0 (reference)	724	95	95/724 (13.1)	-	-	-	-
1 - 50	69	7	7/69 (10.1)	0.75 (0.33, 1.68)	0.482	0.68 (0.29, 1.68)	0.366
51 - 100	26	5	5/26 (19.2)	1.58 (0.58, 4.28)	0.372	1.14 (0.39, 3.28)	0.814
>100	22	8	8/22 (36.4)	3.78 (1.55, 9.26)	0.004	2.53 (0.91, 7.06)	0.076
G3+							
0 (reference)	498	29	29/498 (5.8)	-	-	-	-
1 - 50	30	4	4/30 (13.3)	2.49 (0.81, 7.61)	0.110	2.20 (0.70, 6.86)	0.176
51 - 100	4	0	0/4 (0.0)	-	-	-	-
>100	7	1	1/7 (14.3)	2.70 (0.31, 23.1)	0.366	2.20 (0.25, 19.8)	0.481
All							
0 (reference)	1,222	124	124/1,222 (10.2)	-	-	-	-
1 - 50	99	11	11/99 (11.1)	1.11 (0.58, 2.13)	0.761	0.94 (0.47, 1.85)	0.850
51 - 100	30	5	5/30 (16.7)	1.77 (0.67, 4.71)	0.252	1.13 (0.40, 3.14)	0.822
>100	29	9	9/29 (31.0)	3.98 (1.78, 8.94)	0.001	2.54 (1.03, 6.25)	0.043

† adjusted for maternal age, number of scheduled interventions received, use of bednet, pregnancy season and socioeconomic status

Table 6.4 Peripheral mRDT performance by placental parasite density, stratified by gravidity

Placental parasite density (n/ 500 RBCs)	Total positives by reference test	Positive by RDT	Sensitivity (95% C.I)
G1+2			
1 - 50	26	18	18/26, 69.2% (48.2, 85.7%)
51 - 100	18	12	12/18, 66.7% (41.0, 86.7%)
>100	21	14	14/21, 66.7% (43.0, 85.4%)
G3+			
1 - 50	6	3	3/6, 50.0% (11.8, 88.2%)
51 - 100	4	3	3/4, 75.0% (19.4, 99.4%)
>100	2	2	2/2, 100.0% (15.8, 100.0%)
All			
1 - 50	32	21	21/32, 65.6% (46.8, 81.4%)
51 - 100	22	15	15/22, 68.2% (45.1, 86.1%)
>100	23	16	16/23, 69.6% (47.1, 86.8%)

6.3 Discussion

The utility of RDTs in malaria control has largely been targeted toward the diagnosis of symptomatic malaria to guide initiation of appropriate treatment (141). Consequently the vast majority of studies that have evaluated the performance of RDTs have been within the context of symptomatic malaria, which is usually accompanied by higher peripheral parasite densities than among asymptomatic women (386, 387) . Within this regard, RDTs, more especially combination HRP2/pLDH, have demonstrated very high performance characteristics (293, 388, 389). Though a key susceptible group for malaria infection, the evaluation of RDT performance in pregnant women has largely remained limited (390) but has demonstrated equal reliability to guide malaria treatment as in non-pregnant populations (382, 391, 392). The predominant localization of the biomass of *P.falciparum* malaria in the placenta during the second and third trimesters of pregnancy presents a challenge for the detection of infection sequestered in the placenta by LM but an opportunity for the utility of RDTs. Most studies that have examined the performance of RDTs against this phenomenon have used PCR as the reference test (382). This study is one of the few to date that have compared RDT performance against active placental malaria by placental histology as the reference test for the diagnosis of malaria in pregnancy.

The results of the current study show a high proportion of predominantly *P.falciparum* asymptomatic peripheral and placental malaria infections at delivery. This is in line with results from previous studies (393). Furthermore, the present observations suggest, similar to prior studies, that a large proportion of asymptomatic parasitaemia is largely missed by light microscopy (394, 395). As with many studies, RDT consistently detected more peripheral (283, 396-398) and placental (391, 399, 400) parasitaemia, though this observation differed with other studies that have found RDTs to be inferior to expert microscopy (392) .

Placental histology presents the optimal reference standard for the assessment of the performance of diagnostic tests for active placental malaria (93). The sequestration of viable *P.falciparum* through infected erythrocytes presents a twofold problem for diagnosis of malaria in pregnancy. Firstly, it effectively reduces the density of parasites in the peripheral circulation thus reducing the available concentration of parasites for detection by a diagnostic tests. Secondly, it is likely that the higher concentration of the malaria biomass in the placenta is sufficient enough to perpetrate continued pathophysiological changes leading to adverse pregnancy outcomes. Consequently there is poor detection of malaria biomass causing significant damage to the mother and unborn child. The low performance of otherwise robust peripheral symptomatic malaria diagnostics to detect active placental malaria are therefore not surprising.

An interesting observation in this study was the significant reduction in the sensitivity of RDT and expert microscopy to detect active placental infection from the peripheral blood to the placental blood. It could be postulated that this could be due to the reduced available biomass for detection in the immediate environment of sequestration whereas in the peripheral blood, the biomass would be easier detectable. PCR however proved to be constant in its sensitivity in both milieu, demonstrating significant diagnostic reliability irrespective of sampling site. As such, a PCR method based on peripheral blood is just as good as sampling at site. The observed poor sensitivity of placental blood RDT and LM agree with one known published study to date that used placental histology as the gold standard test. In this study from eastern Sudan in an area of unstable malaria transmission (391) amongst symptomatic pregnant women who would essentially have higher parasite densities, the mean (SD) geometric parasite density was 8,897.8 (1031.0) rings/µl. RDT and PCR had sensitivity 17.5% and 6.5% whilst LM did not detect any malaria. This was despite the same tests having high sensitivities for peripheral malaria with LM as the gold standard. However the study did not evaluate the sensitivity of peripheral blood diagnosis for active placental malaria nor did it compare whether sensitivity differed between the peripheral and placental blood. The findings of the current study

demonstrate higher performance indices, independent of prevalence, than the findings from the study from Sudan. This may lie in the fact that in unstable areas of transmission, pregnant women have poorly developed anti-malarial immunity and therefore symptomatic malaria is easily induced at lower parasite densities. This is opposed to the setting of the current study where though asymptomatic, pregnant women harbored higher parasite densities.

This analysis also sought to elucidate if there was a relationship between placental infection density and the performance of peripheral blood RDT. By adopting the placental parasite density from the placental impression smear as a proxy measure of the density of sequestered placental malaria, there was a strong association between the density of infection and sensitivity of RDT. In addition, it was shown that the risk of LBW with active PM was determined to be significant at >100 parasites/ 500 sequestered RBCs.

The study was not without limitations. The greater test performance by RDTs compared to expert microscopy may be argued to be as a result of poor microscopy technique. Though the microscopy methods employed a robust protocol to ensure quality assurance with a second and third microscopist employed to verify results of the first reader, poor microscopy results may have still emanated from the absence of verification of readings by the lack of external quality assessment as independent assessment of the quality of microscopy. Secondly, the analysis was likely underpowered due to the small number of events of interest in respective analyses as evident by the large confidence intervals in some cases.

From these results, HRP2/pLDH combination RDTs miss a burden of malaria infection in pregnancy after establishment of the placenta due to poor sensitivity to detect parasiteamia sequestered in the placenta. More consistent and sensitive diagnostics methods would therefore be required to catch this missed burden of parasiteamia if screening were to be an effective tool for the control of malaria related morbidity in pregnancy.

7 CHAPTER SEVEN: GENERAL DISCUSSION AND CONCLUSION

"I never quit until I get what I'm after. Negative results are just what I'm after. They are just as valuable to me as positive results." – Thomas A. Edison

An estimated US$ 2.7 billion in international and domestic funding was directed toward the global effort to eliminate malaria in 2013 (24). These efforts have contributed to an overall 30% drop in malaria incidence globally from 2000 to 2013, with Africa registering 4% above the global average decrease in incidence over the same period, signifying the great impact of current efforts in Africa. It is through these concerted efforts that the Millennium Development Goal 6, target C (halted and begun to reverse the incidence of malaria by 2015) has been attained. However, as funding commitments remain below the US$ 5.1 billion benchmark required to successfully control and eliminate malaria, it is apparent that malaria will remain a significant public health problem in the current 94 countries registered with the WHO as having ongoing malaria transmission.

Despite the general penetration of interventions for the control of malaria in Africa having registered significant improvements with an estimated universal coverage of 49% of all people at risk of malaria accessing an insecticide treated bed-net, the uptake of Intermittent Preventative Treatment with Sulphadoxine-Pyrimethamine (IPTp-SP), an essential intervention specifically tailored to pregnant women, remains extremely low. A staggering 42.8% of 35 million pregnant women at risk of malaria are reported not to have received a single dose of preventative treatment for malaria in 2013 (24) despite the continued proven efficacy of Sulphadoxine-Pyrimethamine against alternative regimens, in the context of growing SP resistance. Given the simplicity of implementation of IPTp-SP but the surprising low uptake, it is imperative that any strategy to replace IPTp-SP should not only have superior efficacy and safety, but also be cost effective, acceptable and scalable.

7.1 The efficacy of intermittent screening and treatment for the control of malaria in pregnancy

The concept of intermittent screening and treatment for the control of malaria in pregnancy is based on the assumptions that;

a) Screening detects parasitaemia that would result in adverse pregnancy outcomes if left unchecked.
b) Any parasitaemia not detected would not pose a risk on adverse pregnancy outcomes,
c) By the time of detection of significant levels of parasitaemia, pathological processes that lead to adverse pregnancy outcomes have not been fully established,
d) Treatment with an efficacious antimalarial would halt progression of detected parasitaemia to a critical mass and thus reduces the risk of infections resulting in the irreversible pathophysiological processes that lead to adverse birth outcomes and
e) The post treatment prophylactic effect would sufficiently retard any reinfection and thus reducing therisk of malaria mediated pathophysiological processes that adversely affect the pregnancy until the next antenatal visit.

The findings of the efficacy and safety trial comparing ISTp-DP against IPTp-SP have demonstrated that ISTp-DP is not superior to IPTp-SP in an area of moderate to high malaria transmission with near saturation of quintuple SP mutations and low level (1.5%) sextuple mutants. Further analyses have suggested this lack of superiority may be as a result of a contravention in the earlier assumptions regarding ISTp as outlined below.

7.1.1 Asymptomatic *P.falciparum* infection in pregnancy and diagnostic reliability of RDTs

Results from Chapter 5 have shown that asymptomatic infections have a significant bearing on pregnancy outcomes, however, asymptomatic sub-RDT infections (i.e. those missed by RDTs) appear to have a limited influence on most pregnancy outcomes except for preterm delivery. The majority of patent asymptomatic RDT infections during pregnancy were detected at enrolment (69.9%, N = 299) with most women experiencing only 1 episode (91.3%, N = 275) through the course of pregnancy. This seems to suggest that by the time of presentation for their first antenatal visit,

most women have already experienced significant parasitaemia with possibly irreversible consequences to pregnancy outcomes.

As such exposure reduction interventions in early pregnancy would be required in order to avoid pregnancies from adopting a trajectory toward unfavorable outcomes, an effect which cannot be addressed in light of current practice as SP is contraindicated in the first trimester when malaria infection is greatest in pregnancy. Further studies would be required to substantiate the effect of malaria infection in the first trimester and the impact of intervening at this time point of later pregnancy outcomes.

Despite the current lack of safe chemo-prevention strategies to protect pregnancies at risk in endemic areas in the first trimester, LLITNs provide a strategic reprieve in protecting early pregnancies from the complications of malaria infection as they have been shown to reduce the incidence of parasitaemia and clinical malaria in pregnancy, independent of use of IPTp-SP (161). With only 30% of households having an ITN for people who slept in the household, and 62% of pregnant women reported to having slept under an ITN the night before the Malaria Indicator Survey (187), there is a significant gap in coverage of utilisation of ITNs in early pregnancy (proxy by the proportion of households having an ITN the for people who slept in the household the night before), prior increased utilization upon initiation of antenatal visits where LLITNs are provided. This signifies that active distribution of LLITNs at the community level would provide wider coverage and protection of early pregnancies prior to the initiation of antenatal care. The current local programmatic drive toward universal LLITN coverage not only tackles population level transmission reduction but also, by virtue of providing wider coverage beyond recognised target high risk groups, also provides protection to not yet identified high risk groups such as pregnant women in the first trimester which will reduce the burden of infection by the first antenatal visit and the adverse effects on pregnancy that would have either wise been set in motion prior to targeted interventions that would be accessed from the first antenatal visit.

7.1.2 Implications of the performance of malaria Rapid Diagnostic Tests for the control of pregnancy associated malaria due to placental sequestration

The very low sensitivity of peripheral blood RDTs to detect active placental infection as shown in Chapter 6 implies that such localized infections largely remain undetected through pregnancy. As such the adverse localized effects of placenta sequestration proceed unfettered in screening approach with an RDT. The role of uncleared parasitaemia and the advantage of an efficacious antimalarial has been demonstrated in a recent publication from work conducted in western Kenya where dihydroartemisinin-piperaquine was used for IPTp, showing significantly lowered risks of both peripheral and placental infections than sulphadoxine-pyrimethamine (401), clearly showing that efficacious drugs administered appropriately clear the adverse effects of low density infections by treating peripheral infection so preventing seed to the placenta or clearing already sequestered infections in the placenta.

7.2 Sulphadoxine-Pyrimethamine: Is there more to what meets the eye?

Sulphadoxine-Pyrimethamine consists of a sulfonamide antibiotic (Sulphadoxine) and antiprotozoal (Pyrimethamine) components. It is closely related to the broad spectrum antibiotic drug, Trimethoprim-Sulfamethoxazole (Cotrimoxazole) which has similar constitutional moieties and is used for the prevention of bacterial and protozoal opportunistic infections in HIV infection. Given the relatedness of the two compounds and the dual efficacy of Cotrimoxazole on bacterial infections and malaria, it may be postulated that SP exhibits similar antibiotic effects in addition to its antimalarial properties.

The antibacterial effects of SP in pregnancy have not been subject to full investigation and literature is sparse. The intrinsic antibacterial property of SP against bacterial pathogens common in pregnancy has been previously explore in-vitro and was shown to be highly active against several pathogens (344). There is a paucity of studies that have sought to evaluate the in-vivo efficacy of SP

on ascending genitourinary tract infections in pregnancy or the non-malarial placental biome of the placenta at delivery in order to characterize the importance of non-malarial infections of the placenta on fetal outcomes and neonatal survival. Only one recently published study from Gabon sought to establish the in-vivo efficacy of SP to reduce the prevalence of group B Streptococcus (GBS) in pregnant women (402) the results of which showed that SP did not reduce GBS colonization in-vivo. In this efficacy study, it may be indeed possible that in addition to the longer treatment malaria prophylaxis with IPTp-SP due to more frequent dosing (403), there was an additional beneficial antibacterial treatment effect at the level of the placenta through efficacy on bacterial biomes other than GBS.

7.3 Intermittent Screening and Treatment or Intermittent Testing and Treatment?

The concept of screening as a strategy for the reduction of disease associated morbidity and mortality is based on the potential benefits of early detection of asymptomatic preclinical disease or disease precursors and administration of an effective intervention. Accordingly, the ten point Wilson-Jungner criteria for appraising the validity of a screening programme is essential in considering the viability of a screening strategy (323) though recent alternatives have been proposed after a review of the Wilson-Jungner criteria in the current scientific dispensation (324-327).

Within the context of malaria prevention in pregnancy, the lack of a superior effect in preventing adverse pregnancy outcomes as demonstrated in this trial may be partially explained by a breach in the consideration of Wilson-Jungner criteria in this application.

The concept of screening for malaria infection in pregnancy meets 6 of the proposed criteria:

Criteria 1: Malaria in pregnancy is a recognised public health problem in tropical and mostly underdeveloped countries with devastating consequences for the pregnant woman, unborn child, and early childhood survival and development (2, 96).

Criteria 2: Current WHO recommendations for the treatment of symptomatic malaria infection are with ACTs. The concept of treatment of asymptomatic infection with the IPTp-SP strategy proves that treating presumed asymptomatic infections are beneficial to pregnancy outcomes. The use of an ACT to clear asymptomatic infection in the current environment of declining SP efficacy is therefore a rational alternative.

Criteria 3: In accordance with WHO recommendations, focused antenatal care at all cadres of health facilities provides opportune access points for interventions in pregnancy and favorable outcomes (404). However, as observed with IPTp-SP, several factors interplay that hinder access to FANC services by pregnant women (405, 406).

Criteria 8: As a strategy, it is agreed that there is a dire need for alternative preventive interventions for malaria infection in pregnant women and as such any infected pregnant women is a candidate for treatment.

Criteria 9: Costs of screening for malaria in pregnancy include the cost of RDTs, equipment, personal training, test administration time and space. In light of the reduced efficacy of IPTp-SP, a screening strategy that would be superior in malaria prevention would be cost effective.

Criteria 10: Intermittent screening and treatment is based on routine screening at predefined intervals over the course of the pregnancy until delivery in order to detect pre-effective infections for treatment.

The following failures in the remaining 4 criteria present the limitations of IST and propose further considerations in order to make ISTp more appropriate for the intended purposes:

Criteria 4: The pathophysiology of malaria in pregnancy and how pathological processes are related to adverse birth outcomes are poorly understood (407, 408). As such, it is not possible to define a reliable detectable preclinical phase (DPCP) of disease.

Criteria 5: In light of 4 above, an appropriate and cost-effective test cannot be recommended. By default, it may be postulated that the most sensitive diagnostic test for malaria infection would be

the best bet. However, as demonstrated in Chapter 6, PCR suffers from a considerable limitation in sensitivity to diagnose infection when parasites sequester in the placenta, which may effectually be the DPCP.

Criteria 6: RDTs have been widely accepted and have changed the landscape for appropriate case management of malaria. RDTs were designed for the diagnosis of symptomatic malaria to guide appropriate treatment administration. From Chapter 5, it is evident that RDTs have limitations when used as a screening tests for diagnosing asymptomatic parasitaemia. More sensitive assays that may be applied in field settings such as LAMP may be more promising for detecting low level asymptomatic infections (362, 409-411).

Criteria 7: The natural history of malaria in pregnancy is not fully understood. Despite many studies evaluating effects in the second and third trimester, pathophysiological processes of malaria infection in the first trimester have not been sufficiently explored.

Findings from this work suggest that IST with current RDTs detect relevant levels of parasitaemia associated with adverse birth outcomes. As such the current strategy may be termed "intermittent diagnosis and treatment" since no preclinical stage of disease can be detected and treatment does not necessarily seem to thwart the progression to adverse pregnancy outcomes.

Despite these limitations, an effective screening strategy holds great potential for value addition to antenatal care as it could be easily be integrated into current existing screening strategies in pregnancy such as HIV and syphilis, with the potential to integrate other point of care diagnostics for reproductive tract infections such as chlamydia, in a compact microarray or panel. This benefit would be further potentiated if a screening strategy were to be implemented in the first trimester of pregnancy.

7.4 Cost effectiveness, acceptability and scalability

Ancillary studies to the main efficacy trial, which have not been presented in this thesis, were conducted to determine the cost effectiveness and acceptability of ISTp as a strategy. Evaluation of the efficacy results from the main trial showed that ISTp was not a cost effective strategy. Significant cost drivers were the RDTs and cost of dihydroartemisinin-piperaquine and only a dramatic loss in the efficacy of SP would turn the cost benefit ratio in favor of ISTp-DP.

Findings of the acceptability study identified that although obstacles to the successful implementation of ISTp-DP were acknowledged by health workers and pregnant women, overall both groups considered ISTp as an acceptable alternative to IPTp-SP. The main obstacles to acceptability for health workers were concerns over resources, stock and adherence to DHA-PQ. Pregnant women were generally receptive to ISTp, providing they are able to attend ANC and their expectations were met. IST demonstrated an ability to be easily integrated into the pre-existing antenatal structure and had potential to be scaled up as a policy.

7.5 Recommendations

Local transmission dynamics and drug resistance patterns are major determinants of appropriate strategies for the control of malaria transmission and infection during pregnancy (412). As such, one size will not fit all. Further to ecological factors determining the force of infection in any given setting, the implementation of programmes geared toward malaria eradication (413) will influence at what point strategy changes for malaria control in pregnancy would be appropriate (414) so as to be in tandem with the changing transmission landscape.

7.5.1 Intermittent screening and treatment: "To be or not to be….?"

As shown by findings in chapter 5 and 6, the utility of ISTp is limited by the reduced ability of RDTs to detect pre-patent levels of asymptomatic parasitaemia in the peripheral blood, especially

during the course of subsequent antenatal visits (Chapter 5), and sequestered parasitaemia in the placenta that would warrant treatment (Chapter 6). This is further compounded by the fact that the intervention was implemented after the most vulnerable period to infection i.e. the first trimester where it may have had a more significant impact.

Screening however may not be a totally irrelevant tool for malaria control in pregnancy. Within the current treatment recommendations by the WHO advising for the cautious use of ACTs solely for treatment purposes, screening would detect relevant levels of parasitaemia that would warrant treatment. Based on results from chapter 5, asymptomatic patent infection in pregnancy could warrant consideration as an indication for treatment in line with WHO recommendations for the use of ACTs.

Of note was the much higher overall prevalence of patent and sub-patent infections and the better performance of mRDTs during the initial booking visit, especially in primigravidae. Given the dependency of gestational timing on reliability of RDTs to detect parasitaemia, IST with an ACT may be employed as part of hybrid strategies relevant to local transmission settings within the context of very high SP resistance, and in the absence of better alternatives such as IPTp with alternative antimalarials such as DP that may require a further 2 to 3 years of research. In low transmission settings, screening may be employed as a 'single screen and treat' (SST) strategy as the risk of subsequent patent infection was shown to be much lower during progressive antenatal visits, as is the national policy in Indonesia (189). In high transmission settings, intermittent screening would be employed as part of a mixed screening and presumptive treatment model as the risk of infection remains high in such settings, with women requiring constant chemo-protection. In this approach, women would be screened at each antenatal visit and treated with an ACT if RDT positive or given SP if RDT negative. Such a hybrid strategy was recently introduced in Tanzania, and has been used in parts of Kenya, albeit haphazardly, for some years now.

7.5.2 Alternative strategies

Malaria interventions for the control of malaria in pregnancy are strongly dependent on the broader transmission dynamics in the population and drug resistance patterns. As such, due to the variability in transmission and implementation of malaria eradication programmes between endemic countries, no one size will fit all and individual policies must be tailored accordingly.

The results from this study strongly suggest that in areas of moderate to high transmission, a presumptive treatment approach with an efficacious antimalarial would be best suitable. However, as previously highlighted, to date there are a limited number of efficacious drug options well placed to replace SP for IPTp. However, the efficacy, safety and tolerability of dihydroartemisinin-piperaquine has made it a potential candidate for use as IPTp.

A study evaluating IPTp-DP in Kenya has been recently completed, the results of which are pending publication (238). A previous study evaluating monthly IPT with DP in Ugandan children displayed remarkably results in reducing the incidence of clinical malaria, asymptomatic parasitaemia and anaemia (415) demonstrating the great potential this strategy may have in pregnancy and improving maternal and fetal outcomes.

A significant draw back with any intervention involving DP is the three day course required for the administration of treatment. In light of treatment most likely to be taken at home over three days, there is the concern that adherence may be poor and treatment courses not completed which could result in sub-optimal plasma concentrations. Though this in itself may not warrant a significant fear of the development of drug resistance as pregnant women form a small proportion of the population, the storage and sharing of drugs in communities is not uncommon (416, 417) which could likely perpetuate sub-optimal drug levels in the larger population and drive the development of artemisinin drug resistance, similar to antibiotic resistance (418).

In areas where the prevalence of SP mutations is low, irrespective of transmission intensity, IPTp-SP may still be considered a viable and cost effective strategy. The results from this ISTp-DP

efficacy study demonstrate that IPTp-SP continues to be an effective in the absence of more cost effective alternatives in areas of near saturation of the quintuple SP mutation, low prevalence of the sextuple mutants and moderate to high malaria transmission. Daily cotrimoxazole prophylaxis may be considered in settings with a high prevalence of sextuple mutants as cotrimoxazole prophylaxis remains efficacious against malaria infection and is not hindered by SP resistance mutations despite concerns of cross-resistance.

7.5.3 Molecular monitoring of high level SP resistance markers

The additional *dhps* A581G mutation to the quintuple mutation confers very high grade resistance and near total SP treatment failure. It would thus be imperative that monitoring of this mutation in addition to the K540E marker for quintuple mutation be included as part of routine malaria surveys to aptly provide indication for possible policy changes to ensure adequate protection of pregnant women and their off-spring from the consequences of malaria infection during pregnancy.

7.6 Current and Future research

7.6.1 The role of the bacterial placental biome on pregnancy outcomes and potential effect of adjunct antibacterial therapy in malaria interventions in pregnancy

Intercellular bacterial infections have been recognised to contribute substantially to adverse pregnancy outcomes and neonatal infections (419). Despite evidence of a synergistic relationship between malaria and some bacterial infections in other settings, this has not been fully explored in pregnancy, especially in settings where malaria and bacterial infections overlap. Studies that have co-opted antibacterial regimens as part of combination treatment with an antimalarial have seldom investigated the effect on the bacterial biome of the placenta and whether this contributed to the observed pregnancy outcomes.

In a placebo-controlled trial of oral azithromycin for the prevention of preterm delivery in southern Malawi, routine prophylaxis did not have a significantly different effect on preterm birth, birthweight maternal anaemia, malaria infection or perinatal mortality (420). No placental histology was reported from this trial. Contrary to these findings, two randomized control clinical trials have found the use of azithromycin with SP to have beneficial effects on pregnancy outcomes. An earlier trial from Malawi found reduced risk of preterm birth and low birthweight with monthly SP and two prophylactic doses of azithromycin (421). A more recent trial from Papua New Guinea found that the addition of Azithromycin to IPTp-SP reduced preterm delivery and low birthweight with a reduction in the carriage of gonorrhoea as well (422). The use of azithromycin was associated with reduced carriage of other bacterial pathogens (423).

In an expert review on combination therapy for the prevention of malaria and genitourinary tract infections in pregnancy, the addition of an antibacterial to an antimalarial, such as azithromycin with chloroquine would be essential to address the adverse effects of both malaria and bacterial infections on pregnancy outcomes (230), particular in the early pregnancy when most regimens are contraindicated. With these results, the consideration of adding adjunct antibiotic therapy to a promising routine intervention for the prevention of malaria in pregnancy would be warranted.

7.6.2 Intermittent presumptive treatment in pregnancy with an Artemisinin combination therapy in the first trimester

The limited alternatives to replace SP for IPTp has prompted great interest in the use of ACTs for malaria prevention in pregnancy due to their high treatment efficacy, post treatment prophylaxis, safety and tolerability (172, 424). The greatest burden of malaria infection in pregnancy has been shown to occur in the first trimester commonly between 9 and 16 weeks of gestation when most interventions have been contraindicated save for chloroquine and azithromycin. A growing body of

evidence appears to suggest that ACTs do not confer any higher risk of adverse events in pregnancy when administered in the first trimester (171, 425, 426).

More evidence is required on the safety of first trimester administration of ACTs as well as the effect of repeated dosing in pregnancy as current evidence remains small. Results from a repeat fix dose treatment study in children from Uganda with Artesunate-Amodiaquine (ASAQ) and Artemether-Lumefantrine (AL) have shown no remarkable adverse effects and are considered safe (427). The major concern with repeated dosing of Dihydroartemisinin-piperaquine is with the risk of prolonged QT interval (180, 428) that may lead to *torsade's de pointes* (TdP) after successive accumulation of Piperaquine in a dose dependant manner (429) via blockade of the I_{Kr} (rapidly depolarising potassium ion) channels. However, in several single dose studies that have noted prolonged QT intervals following DP administration, such prolongations have been transient and resolved by day 7 post treatment with no deaths ascribed to TdP (430, 431).

The potential ramifications of proven safety of first trimester administration as well as multiple dose administration would enable the use of an efficacious intervention against malaria in pregnancy at the period when pregnancies are most vulnerable to malaria infection. In addition, the provision of chemo-intervention, which has been viewed as a motivational factor for ANC attendance as part of keeping the pregnancy safe from malaria (406, 432, 433) could encourage early attendance.

7.6.3 Chemoprevention for HIV infected pregnant women and interactions between ACTs and ARVs in pregnancy

Pregnant women with HIV infection are at an increased risk of malaria infection and more likely to suffer from untoward pregnancy outcomes due to the combined and synergistic consequences of HIV and malaria infection (51, 434, 435). Current recommendations for the control of malaria in pregnancies complicated by HIV infection exclude IPTp-SP as women receive daily cotrimoxazole prophylaxis for the prevention of opportunistic infections which provides additional protection from malaria infection (436, 437). Furthermore, as cotrimoxazole and SP are both

sulphonamide drugs, co-administration presents an increased risk for sulphonamide derived side effects especially severe cutaneous reactions (438-440).

Due to their related mode of action as antimalarials by similarly inhibiting the *dhfr* (via Trimethoprim and Pyrimethamine components)and *dhp*s (via Sulfamethoxazole and Sulphadoxine) enzymes essential to parasite metabolism (441), rising SP resistance has raised concerns regarding the continued efficacy of daily cotrimoxazole for prevention of malaria related morbidity in HIV infected pregnant women and as a source of sustained drug pressure fueling continued selection pressure for SP resistant parasites. Despite results from in-vitro studies suggesting cross resistance (442), findings from previous field studies have demonstrated this not to be the case (443, 444) and cotrimoxazole chemoprophylaxis remains efficacious (445, 446). A recent published multicenter study however, demonstrated that with the addition of a IPT administered Mefloquine to daily cotrimoxazole prophylaxis, improved malaria prevention but was associated with higher viral loads and mother to child transmission of HIV (237).

Despite revised WHO recommendations to initiate ARTs in pregnant and breast-feeding women irrespective of CD4 count or WHO staging (447), cotrimoxazole prophylaxis has remained a key policy recommendation for HIV management amongst pregnant women especially where malaria transmission remains moderate to high (malaria prevalence greater than 10% among children aged 2-9 years old). Given evidence of the additional benefit of malaria prevention by the inclusion of a non-SP IPTp regimen, evaluating the use of an ACT for IPTp in HIV infected pregnant women would be warranted. Little is known about the interactions of ACTs and ARVs and a study to evaluate drug-drug interactions and the impact on infection and pregnancy outcomes is important.

7.7 Overall conclusion and recommendations

The findings of the work undertaken in this thesis strongly indicate that intermittent screening and treatment for the control of malaria in pregnancy in an areas of moderate to high

malaria transmission and near saturation of high grade SP resistance is not a suitable alternative to intermittent preventive treatment with SP for the control of malaria in pregnancy, and may even predispose to adverse maternal and fetal outcomes. These results are consistent with similar trials conducted in Western Kenya and West Africa. The failure of ISTp may be attributed to the infrequency of screening (approximately monthly) to pick up significant infections in a high transmission setting and from the failure of the current generation of malaria RDTs to detect chronic low-level parasitemia that are only detectable by PCR, allowing parasites to persist from weeks to months in the placenta. Furthermore, the results suggest that SP remains at least partially effective at improving birthweights and preventing pregnancy loss. The surprisingly resilient beneficial effect of SP may reflect a partial antimalarial effect that may be insufficient to clear parasites (treatment effect), but enough to provide some prophylactic benefit in these semi-immune women by suppressing parasite densities in the placenta (rather than clearing them) to sufficiently low levels to reduce in part, the harmful effects of malaria infection in pregnancy. Furthermore, it is possible that the residual beneficial effect of SP, could also reflect its broad antimicrobial activity conferring some protection against undetected bacterial infections, particularly gram-positive bacteria (448), or due to an effect on either the gut or vaginal microbiota.

In light of the consistent failure of ISTp to demonstrate any beneficial effect on pregnancy outcomes, it is strongly recommended that ISTp should not be considered as a viable strategy to replace IPTp-SP for the control of malaria in pregnancy in areas of moderate to high transmission and low to high SP resistance. However, within settings of low transmission or near elimination, screening may prove to be a viable and cost effective alternative. The current resilience of SP offers a window of opportunity for the continued use of IPTp-SP until superior interventions are developed and verified, though within a limited time period owing to the rapid emergence of more aggressive resistant strains characterised by the A581G mutation.

Within this window of opportunity, it is essential to monitor how fast it would be closing by incorporating molecular monitoring of the A581G mutation. Furthermore, as it is apparent from this trial that within a setting of moderate to high malaria transmission, continuous protection from infection be provided to pregnant women, it is imperative that not only alternative drug regimens for IPTp be identified and verified for use in both HIV-seronegative and –seropositive women, but also that there is a need for enhanced efforts to accelerate malaria transmission reduction through increased coverage of current vector control strategies, and innovative reservoir and vector elimination interventions to reduce the transmission burden at the larger population level.

8 REFERENCES

1. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. PLoS Med. 2010;7(1):e1000221.

2. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. The Lancet Infectious Dis. 2007;7(2):93-104.

3. Kayentao K, Garner P, van Eijk AM, Naidoo I, Roper C, Mulokozi A, et al. Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in Africa: systematic review and meta-analysis. JAMA. 2013;309(6):594-604.

4. McClure EM, Goldenberg RL, Dent AE, Meshnick SR. A systematic review of the impact of malaria prevention in pregnancy on low birth weight and maternal anemia. Int J Gynaecol Obstet. 2013;121(2):103-9.

5. Rijken MJ, De Livera AM, Lee SJ, Boel ME, Rungwilailaekhiri S, Wiladphaingern J, et al. Quantifying low birth weight, preterm birth and small-for-gestational-age effects of malaria in pregnancy: a population cohort study. PLoS One. 2014;9(7):e100247.

6. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. Clin Microbiol Rev. 2004;17(4):760-9.

7. Guyatt HL, Snow RW. Malaria in pregnancy as an indirect cause of infant mortality in sub-Saharan Africa. Trans R Soc Trop Med Hyg. 2001;95(6):569-76.

8. McDonald CR, Elphinstone RE, Kain KC. The impact of placental malaria on neurodevelopment of exposed infants: a role for the complement system? Trends Parasitol. 2013;29(5):213-9.

9. Moya-Alvarez V, Abellana R, Cot M. Pregnancy-associated malaria and malaria in infants: an old problem with present consequences. Malar J. 2014;13:271.

10. Walther B, Miles DJ, Crozier S, Waight P, Palmero MS, Ojuola O, et al. Placental malaria is associated with reduced early life weight development of affected children independent of low birth weight. Malar J. 2010;9:16.

11. WHO. WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulphadoxine-pyrimethamine (IPTP-SP). Geneva, Switzerland: World Health Organization, 2014.

12. WHO. Guidelines for the treatment of malaria: second edition. Geneva: World Health Organization; 2010.

13. WHO. A strategic framework for malaria prevention and control during pregnancy in the African Region. Brazzaville: World Health Organization Regional Office for Africa, 2004.

14. ter Kuile FO, van Eijk AM, Filler SJ. Effect of sulfadoxine-pyrimethamine resistance on the efficacy of intermittent preventive therapy for malaria control during pregnancy: a systematic review. JAMA. 2007;297(23):2603-16.

15. Kayentao K, Garner P, Maria van Eijk A, et al. Intermittent preventive therapy for malaria during pregnancy using 2 vs 3 or more doses of sulfadoxine-pyrimethamine and risk of low birth weight in africa: Systematic review and meta-analysis. JAMA. 2013;309(6):594-604.

16. Harrington WE, Morrison R, Fried M, Duffy PE. Intermittent Preventive Treatment in Pregnant Women Is Associated with Increased Risk of Severe Malaria in Their Offspring. PLoS One. 2013;8(2):e56183.

17. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. Intermittent Treatment to Prevent Pregnancy Malaria Does Not Confer Benefit in an Area of Widespread Drug Resistance. Clin Infect Dis. 2011;53(3):224-30.

18. Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, Fried M, et al. Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci. 2009;106(22):9027-32.

19. ter Kuile FO, Larsen DA, Roper C, Koshy G, Taylor SD, Meghna , Eisele T, et al. Impact of sulphadoxine-pyrimethamine resistance on the effectiveness of Intermittent Preventive Therapy for malaria in pregnancy (IPTp) in Africa: A systematic review and meta-analysis. Unpublished.

20. United Nations General Assembly. United Nations Millennium Declaration. Resolution adopted. United Nations, 2000.

21. Sachs J, Malaney P. The economic and social burden of malaria. Nature. 2002;415(6872):680-5.

22. Gallup J, Sachs J. The economic burden of malaria. Am J Trop Med Hyg. 2001;64(1 suppl):85-96.

23. Malaney P, Spielman A, Sachs J. THE MALARIA GAP. Am J Trop Med Hyg. 2004;71(2 suppl):141-6.

24. World Health Organization. World Malaria Report 2014. Geneva: World Health Organization, 2014.

25. Guyatt HL, Snow RW. Impact of Malaria during Pregnancy on Low Birth Weight in Sub-Saharan Africa. Clin Microbiol Rev. 2004;17(4):760-9.

26. Van Geertruyden J-P, Thomas F, Erhart A, D'Alessandro U. THE CONTRIBUTION OF MALARIA IN PREGNANCY TO PERINATAL MORTALITY. Am J Trop Med Hyg. 2004;71(2 suppl):35-40.

27. Roll Back Malaria. The contribution of malaria control to maternal and newborn health 2014 [cited 2015 22 March]. Available from: http://www.rollbackmalaria.org/ProgressImpactSeries/report17.html.

28. Ansell J, Hamilton KA, Pinder M, Walraven GE, Lindsay SW. Short-range attractiveness of pregnant women to Anopheles gambiae mosquitoes. Trans R Soc Trop Med Hyg. 2002;96(2):113-6.

29. Le Hesran JY, Cot M, Personne P, Fievet N, Dubois B, Beyeme M, et al. Maternal placental infection with Plasmodium falciparum and malaria morbidity during the first 2 years of life. Am J Epidemiol. 1997;146(10):826-31.

30. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, Narum DL, et al. Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. PLoS Med. 2009;6(7):e1000116.

31. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, et al. Maternal malaria and gravidity interact to modify infant susceptibility to malaria. PLoS Med. 2005;2(12):e407.

32. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, et al. Placental malaria increases malaria risk in the first 30 months of life. Clin Infect Dis. 2008;47(8):1017-25.

33. Cornet M, Le Hesran JY, Fievet N, Cot M, Personne P, Gounoue R, et al. Prevalence of and risk factors for anemia in young children in southern Cameroon. Am J Trop Med Hyg. 1998;58(5):606-11.

34. van Eijk AM, Ayisi JG, Ter Kuile FO, Misore AO, Otieno JA, Kolczak MS, et al. Malaria and human immunodeficiency virus infection as risk factors for anemia in infants in Kisumu, western Kenya. Am J Trop Med Hyg. 2002;67(1):44-53.

35. Reed SC, Wirima JJ, Steketee RW. Risk factors for anemia in young children in rural Malawi. Am J Trop Med Hyg. 1994;51(2):170-4.

36. Owens Sea. Placental malaria and gravidity interact to modify infant susceptibility to malaria. Arch Dis Child. 2009;91:507-8.

37. UNICEF. New York: United Nations Population Division, & United Nations Statistics Division; 2013 [updated 27 December 2013; cited 2015 04 March]. Available from: http://www.unicef.org/infobycountry/malawi_statistics.html.

38. Emina JBO, Madise N, Kuepie M, Zulu EM, Ye Y. Identifying HIV most-at-risk groups in Malawi for targeted interventions. A classification tree model. BMJ Open. 2013;3(5).

39. Kazembe LN, Kleinschmidt I, Holtz TH, Sharp BL. Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. Int J Health Geogr. 2006;5:41.

40. Mzilahowa T, Hastings IM, Molyneux ME, McCall PJ. Entomological indices of malaria transmission in Chikhwawa district, Southern Malawi. Malar J. 2012;11:380-.

41. Spiers AA MT, Atkinson D, McCall PJ. The malaria vectors of the Lower Shire Valley, Malawi. Malawi Med J. 2002(14):4-7.

42. Mzilahowa T, Ball AJ, Bass C, Morgan JC, Nyoni B, Steen K, et al. Reduced susceptibility to DDT in field populations of Anopheles quadriannulatus and Anopheles arabiensis in Malawi: evidence for larval selection. Med Vet Entomol. 2008;22(3):258-63.

43. Wondji CS, Coleman M, Kleinschmidt I, Mzilahowa T, Irving H, Ndula M, et al. Impact of pyrethroid resistance on operational malaria control in Malawi. Proc Natl Acad Sci U S A. 2012;109(47):19063-70.

44. Lindblade KA, Mwandama D, Mzilahowa T, Steinhardt L, Gimnig J, Shah M, et al. A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. Malar J. 2015;14(1):31.

45. Rantala A-M, Taylor SM, Trottman PA, Luntamo M, Mbewe B, Maleta K, et al. Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malar J. 2010;9:269-.

46. Taylor SM, Antonia AL, Chaluluka E, Mwapasa V, Feng G, Molyneux ME, et al. Antenatal Receipt of Sulfadoxine-Pyrimethamine Does Not Exacerbate Pregnancy-Associated Malaria Despite the Expansion of Drug-Resistant Plasmodium falciparum: Clinical Outcomes From the QuEERPAM Study. Clin Infect Dis. 2012;55(1):42-50.

47. Hochman S, Kim K. The Impact of HIV and Malaria Coinfection: What Is Known and Suggested Venues for Further Study. Interdisciplinary perspectives on infectious diseases. 2009;2009:617954.

48. Brentlinger PE, Behrens CB, Micek MA. Challenges in the concurrent management of malaria and HIV in pregnancy in sub-Saharan Africa. Lancet Infect Dis. 2006;6(2):100-11.

49. van Eijk AM, Ayisi JG, ter Kuile FO, Misore AO, Otieno JA, Rosen DH, et al. HIV increases the risk of malaria in women of all gravidities in Kisumu, Kenya. AIDS (London, England). 2003;17(4):595-603.

50. Keen J, Serghides L, Ayi K, Patel SN, Ayisi J, van Eijk A, et al. HIV impairs opsonic phagocytic clearance of pregnancy-associated malaria parasites. PLoS Med. 2007;4(5):e181.

51. ter Kuile FO, Parise ME, Verhoeff FH, Udhayakumar V, Newman RD, van Eijk AM, et al. The burden of co-infection with human immunodeficiency virus type 1 and malaria in pregnant women in sub-saharan Africa. Am J Trop Med Hyg. 2004;71(2 Suppl):41-54.

52. Ayisi JG, van Eijk AM, Newman RD, ter Kuile FO, Shi YP, Yang C, et al. Maternal malaria and perinatal HIV transmission, western Kenya. Emerg Infect Dis. 2004;10(4):643-52.

53. Mwapasa V, Rogerson SJ, Molyneux ME, Abrams ET, Kamwendo DD, Lema VM, et al. The effect of Plasmodium falciparum malaria on peripheral and placental HIV-1 RNA concentrations in pregnant Malawian women. AIDS (London, England). 2004;18(7):1051-9.

54. Briand V, Badaut C, Cot M. Placental malaria, maternal HIV infection and infant morbidity. Ann Trop Paediatr. 2009;29(2):71-83.

55. Inion I, Mwanyumba F, Gaillard P, Chohan V, Verhofstede C, Claeys P, et al. Placental malaria and perinatal transmission of human immunodeficiency virus type 1. J Infect Dis. 2003;188(11):1675-8.

56. Ayouba A, Badaut C, Kfutwah A, Cannou C, Juillerat A, Gangnard S, et al. Specific stimulation of HIV-1 replication in human placental trophoblasts by an antigen of Plasmodium falciparum. AIDS (London, England). 2008;22(6):785-7.

57. Renzaho AM. Mortality rates, prevalence of malnutrition, and prevalence of lost pregnancies among the drought-ravaged population of Tete Province, Mozambique. Prehosp Disaster Med. 2007;22(1):26-34.

58. Gresham E, Byles JE, Bisquera A, Hure AJ. Effects of dietary interventions on neonatal and infant outcomes: a systematic review and meta-analysis. Am J Clin Nutr. 2014;100(5):1298-321.

59. Abu-Saad K, Fraser D. Maternal Nutrition and Birth Outcomes. Epidemiol Rev. 2010;32(1):5-25.

60. Rayco-Solon P, Fulford AJ, Prentice AM. Differential effects of seasonality on preterm birth and intrauterine growth restriction in rural Africans. Am J Clin Nutr. 2005;81(1):134-9.

61. Caulfield LE, Richard SA, Black RE. Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old. Am J Trop Med Hyg. 2004;71(2 Suppl):55-63.

62. Landis SH, Lokomba V, Ananth CV, Atibu J, Ryder RW, Hartmann KE, et al. Impact of maternal malaria and under-nutrition on intrauterine growth restriction: a prospective ultrasound study in Democratic Republic of Congo. Epidemiol Infect. 2009;137(2):294-304.

63. Menendez C, Romagosa C, Ismail MR, Carrilho C, Saute F, Osman N, et al. An autopsy study of maternal mortality in Mozambique: the contribution of infectious diseases. PLoS Med. 2008;5(2):e44.

64. Singh K, Moran A, Story W, Bailey P, Chavane L. Acknowledging HIV and malaria as major causes of maternal mortality in Mozambique. Int J Gynaecol Obstet. 2014;127(1):35-40.

65. William T, Jelip J, Menon J, Anderios F, Mohammad R, Awang Mohammad TA, et al. Changing epidemiology of malaria in Sabah, Malaysia: increasing incidence of Plasmodium knowlesi. Malar J. 2014;13:390.

66. Yusof R, Lau YL, Mahmud R, Fong MY, Jelip J, Ngian HU, et al. High proportion of knowlesi malaria in recent malaria cases in Malaysia. Malar J. 2014;13:168.

67. Barber BE, Bird E, Wilkes CS, William T, Grigg MJ, Paramaswaran U, et al. Plasmodium knowlesi Malaria During Pregnancy. J Infect Dis. 2015;211(7):1104-10.

68. Duffy PE. Plasmodium in the placenta: parasites, parity, protection, prevention and possibly preeclampsia. Parasitology. 2007;134(Pt 13):1877-81.

69. Arango E, Maestre A, Carmona-Fonseca J. [Effect of submicroscopic or polyclonal Plasmodium falciparum infection on mother and gestation product: systematic review]. Rev Bras Epidemiol. 2010;13(3):373-86.

70. Ataide R, Mayor A, Rogerson SJ. Malaria, primigravidae, and antibodies: knowledge gained and future perspectives. Trends Parasitol. 2014;30(2):85-94.

71. Thellin O, Coumans B, Zorzi W, Igout A, Heinen E. Tolerance to the foeto-placental 'graft': ten ways to support a child for nine months. Current opinion in immunology. 2000;12(6):731-7.

72. Piccinni MP. T cell tolerance towards the fetal allograft. J Reprod Immunol. 2010;85(1):71-5.

73. Brabin BJ, Johnson PM. Placental malaria and pre-eclampsia through the looking glass backwards? J Reprod Immunol. 2005;65(1):1-15.

74. Taylor-Robinson AW. Regulation of immunity to Plasmodium: implications from mouse models for blood stage malaria vaccine design. Exp Parasitol. 2010;126(3):406-14.

75. Hamer DH, Mwanakasale V, MacLeod WB, Chalwe V, Mukwamataba D, Champo D, et al. Two-Dose versus Monthly Intermittent Preventive Treatment of Malaria with Sulfadoxine-Pyrimethamine in HIV-Seropositive Pregnant Zambian Women. J Infect Dis. 2007;196(11):1585-94.

76. Fievet N, Moussa M, Tami G, Maubert B, Cot M, Deloron P, et al. Plasmodium falciparum induces a Th1/Th2 disequilibrium, favoring the Th1-type pathway, in the human placenta. J Infect Dis. 2001;183(10):1530-4.

77. Rogerson SJ, Brown HC, Pollina E, Abrams ET, Tadesse E, Lema VM, et al. Placental Tumor Necrosis Factor Alpha but Not Gamma Interferon Is Associated with Placental Malaria and Low Birth Weight in Malawian Women. Infect Immun. 2003;71(1):267-70.

78. Moore JM, Nahlen BL, Misore A, Lal AA, Udhayakumar V. Immunity to placental malaria. I. Elevated production of interferon-gamma by placental blood mononuclear cells is associated with protection in an area with high transmission of malaria. J Infect Dis. 1999;179(5):1218-25.

79. Suguitan AL, Jr., Leke RG, Fouda G, Zhou A, Thuita L, Metenou S, et al. Changes in the levels of chemokines and cytokines in the placentas of women with Plasmodium falciparum malaria. J Infect Dis. 2003;188(7):1074-82.

80. Marsh K, Kinyanjui S. Immune effector mechanisms in malaria. Parasite Immunol. 2006;28(1-2):51-60.

81. Beeson JG, Osier FH, Engwerda CR. Recent insights into humoral and cellular immune responses against malaria. Trends Parasitol. 2008;24(12):578-84.

82. Serghides L, Patel SN, Ayi K, Kain KC. Placental Chondroitin Sulfate A–Binding Malarial Isolates Evade Innate Phagocytic Clearance. J Infect Dis. 2006;194(1):133-9.

83. Rieger H, Yoshikawa HY, Quadt K, Nielsen MA, Sanchez CP, Salanti A, et al. Cytoadhesion of Plasmodium falciparum–infected erythrocytes to chondroitin-4-sulfate is cooperative and shear enhanced. Blood. 2014;125(2):383-91.

84. Fowkes FJ, McGready R, Cross NJ, Hommel M, Simpson JA, Elliott SR, et al. New insights into acquisition, boosting, and longevity of immunity to malaria in pregnant women. J Infect Dis. 2012;206(10):1612-21.

85. Duffy PE, Fried M. Malaria in the pregnant woman. Curr Top Microbiol Immunol. 2005;295:169-200.

86. Mayor A, Rovira-Vallbona E, Machevo S, Bassat Q, Aguilar R, Quinto L, et al. Parity and placental infection affect antibody responses against Plasmodium falciparum during pregnancy. Infect Immun. 2011;79(4):1654-9.

87. Umbers AJ, Stanisic DI, Ome M, Wangnapi R, Hanieh S, Unger HW, et al. Does Malaria Affect Placental Development? Evidence from In Vitro Models. PLoS One. 2013;8(1):e55269.

88. Silver KL, Zhong K, Leke RGF, Taylor DW, Kain KC. Dysregulation of Angiopoietins Is Associated with Placental Malaria and Low Birth Weight. PLoS One. 2010;5(3):e9481.

89. Conroy AL, Silver KL, Zhong K, Rennie M, Ward P, Sarma JV, et al. Complement activation and the resulting placental vascular insufficiency drives fetal growth restriction associated with placental malaria. Cell Host Microbe. 2013;13(2):215-26.

90. Salanti A, Dahlback M, Turner L, Nielsen MA, Barfod L, Magistrado P, et al. Evidence for the involvement of VAR2CSA in pregnancy-associated malaria. J Exp Med. 2004;200(9):1197-203.

91. Parekh FK, Davison BB, Gamboa D, Hernandez J, Branch OH. Placental histopathologic changes associated with subclinical malaria infection and its impact on the fetal environment. Am J Trop Med Hyg. 2010;83(5):973-80.

92. Ismail MR, Ordi J, Menendez C, Ventura PJ, Aponte JJ, Kahigwa E, et al. Placental pathology in malaria: a histological, immunohistochemical, and quantitative study. Hum Pathol. 2000;31(1):85-93.

93. Muehlenbachs A, Fried M, McGready R, Harrington WE, Mutabingwa TK, Nosten F, et al. A Novel Histological Grading Scheme for Placental Malaria Applied in Areas of High and Low Malaria Transmission. J Infect Dis. 2010;202(10):1608-16.

94. Chotivanich K, Udomsangpetch R, Suwanarusk R, Pukrittayakamee S, Wilairatana P, Beeson JG, et al. Plasmodium vivax adherence to placental glycosaminoglycans. PLoS One. 2012;7(4):e34509.

95. Souza RM, Ataide R, Dombrowski JG, Ippolito V, Aitken EH, Valle SN, et al. Placental histopathological changes associated with Plasmodium vivax infection during pregnancy. PLoS Negl Trop Dis. 2013;7(2):e2071.

96. Uneke CJ. Impact of Placental Plasmodium falciparum Malaria on Pregnancy and Perinatal Outcome in Sub-Saharan Africa: II: Effects of Placental Malaria on Perinatal Outcome; Malaria and HIV. Yale J Biol Med. 2007;80(3):95-103.

97. Umbers AJ, Boeuf P, Clapham C, Stanisic DI, Baiwog F, Mueller I, et al. Placental Malaria-Associated Inflammation Disturbs the Insulin-like Growth Factor Axis of Fetal Growth Regulation. J Infect Dis. 2011;203(4):561-9.

98. Muehlenbachs A, Mutabingwa TK, Edmonds S, Fried M, Duffy PE. Hypertension and maternal-fetal conflict during placental malaria. PLoS Med. 2006;3(11):e446.

99. Mockenhaupt FP, Ulmen U, von Gaertner C, Bedu-Addo G, Bienzle U. Diagnosis of placental malaria. J Clin Microbiol. 2002;40(1):306-8.

100. Fried M, Nosten F, Brockman A, Brabin BJ, Duffy PE. Maternal antibodies block malaria. Nature. 1998;395(6705):851-2.

101. Megnekou R, Staalsoe T, Taylor DW, Leke R, Hviid L. Effects of Pregnancy and Intensity of Plasmodium falciparum Transmission on Immunoglobulin G Subclass Responses to Variant Surface Antigens. Infect Immun. 2005;73(7):4112-8.

102. Guyatt HL, Snow RW. The epidemiology and burden of Plasmodium falciparum-related anemia among pregnant women in sub-Saharan Africa. Am J Trop Med Hyg. 2001;64(1-2 Suppl):36-44.

103. Schrier RW, Fassett RG, Ohara M, Martin PY. Pathophysiology of renal fluid retention. Kidney Int Suppl. 1998;67:S127-32.

104. Valore EV, Ganz T. Posttranslational processing of hepcidin in human hepatocytes is mediated by the prohormone convertase furin. Blood Cells Mol Dis. 2008;40(1):132-8.

105. Nemeth E, Ganz T. The role of hepcidin in iron metabolism. Acta Haematol. 2009;122(2-3):78-86.

106. Khalafallah AA, Dennis AE. Iron Deficiency Anaemia in Pregnancy and Postpartum: Pathophysiology and Effect of Oral versus Intravenous Iron Therapy. J Pregnancy. 2012;2012:630519.

107. Perkins DJ, Were T, Davenport GC, Kempaiah P, Hittner JB, Ong'echa JM. Severe Malarial Anemia: Innate Immunity and Pathogenesis. Int J Biol Sci. 2011;7(9):1427-42.

108. Evans KJ, Hansen DS, van Rooijen N, Buckingham LA, Schofield L. Severe malarial anemia of low parasite burden in rodent models results from accelerated clearance of uninfected erythrocytes. Blood. 2005;107(3):1192-9.

109. Oldenborg PA, Zheleznyak A, Fang YF, Lagenaur CF, Gresham HD, Lindberg FP. Role of CD47 as a marker of self on red blood cells. Science (New York, NY). 2000;288(5473):2051-4.

110. Safeukui I, Correas JM, Brousse V, Hirt D, Deplaine G, Mule S, et al. Retention of Plasmodium falciparum ring-infected erythrocytes in the slow, open microcirculation of the human spleen. Blood. 2008;112(6):2520-8.

111. Luxemburger C, McGready R, Kham A, Morison L, Cho T, Chongsuphajaisiddhi T, et al. Effects of Malaria during Pregnancy on Infant Mortality in an Area of Low Malaria Transmission. Am J Epidemiol. 2001;154(5):459-65.

112. Newman RD, Hailemariam A, Jimma D, Degifie A, Kebede D, Rietveld AE, et al. Burden of malaria during pregnancy in areas of stable and unstable transmission in Ethiopia during a nonepidemic year. J Infect Dis. 2003;187(11):1765-72.

113. Adegnika AA, Verweij JJ, Agnandji ST, Chai SK, Breitling LP, Ramharter M, et al. Microscopic and sub-microscopic plasmodium falciparum infection, but not inflammation caused by infection is associated with low birth weight. Am J Trop Med Hyg. 2006;75(5):798-803.

114. Le Hesran JY, Cot M, Personne P, Fievet N, Dubois B, Beyeme M, et al. Maternal placental infection with Plasmodium falciparum and malaria morbidity during the first 2 years of life. Am J Epidemiol. 1997;146(10):826-31.

115. Cot M, Le Hesran JY, Staalsoe T, Fievet N, Hviid L, Deloron P. Maternally transmitted antibodies to pregnancy-associated variant antigens on the surface of erythrocytes infected with

Plasmodium falciparum: relation to child susceptibility to malaria. Am J Epidemiol. 2003;157(3):203-9.

116. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, Narum DL, et al. Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. PLoS Med. 2009;6(7):e1000116.

117. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, et al. Maternal malaria and gravidity interact to modify infant susceptibility to malaria. PLoS Med. 2005;2(12):e407.

118. Kalanda BF, Verhoeff FH, Chimsuku L, Harper G, Brabin BJ. Adverse birth outcomes in a malarious area. Epidemiol Infect. 2006;134(3):659-66.

119. Watson-Jones D, Weiss HA, Changalucha JM, Todd J, Gumodoka B, Bulmer J, et al. Adverse birth outcomes in United Republic of Tanzania--impact and prevention of maternal risk factors. Bulletin of the World Health Organization. 2007;85(1):9-18.

120. Verhoeff FH, Le Cessie S, Kalanda BF, Kazembe PN, Broadhead RL, Brabin BJ. Post-neonatal infant mortality in Malawi: the importance of maternal health. Ann Trop Paediatr. 2004;24(2):161-9.

121. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, et al. Placental malaria increases malaria risk in the first 30 months of life. Clin Infect Dis. 2008;47(8):1017-25.

122. Adegnika AA, Kohler C, Agnandji ST, Chai SK, Labuda L, Breitling LP, et al. Pregnancy-associated malaria affects toll-like receptor ligand-induced cytokine responses in cord blood. The J Infect Dis. 2008;198(6):928-36.

123. Asante KP, Owusu-Agyei S, Cairns M, Dodoo D, Boamah EA, Gyasi R, et al. Placental malaria and the risk of malaria in infants in a high malaria transmission area in ghana: a prospective cohort study. J Infect Dis. 2013;208(9):1504-13.

124. Apinjoh TO, Anchang-Kimbi JK, Mugri RN, Njua-Yafi C, Tata RB, Chi HF, et al. Determinants of Infant Susceptibility to Malaria During the First Year of Life in South Western Cameroon. Open Forum Infect Dis. 2015;2(1).

125. Bardaji A, Sigauque B, Sanz S, Maixenchs M, Ordi J, Aponte JJ, et al. Impact of malaria at the end of pregnancy on infant mortality and morbidity. J Infect Dis. 2011;203(5):691-9.

126. Steketee RW. Pregnancy, Nutrition and Parasitic Diseases. J Nutr. 2003;133(5):1661S-7S.

127. Verhoef H, West CE, Veenemans J, Beguin Y, Kok FJ. Stunting may determine the severity of malaria-associated anemia in African children. Pediatrics. 2002;110(4):e48.

128. Deen JL, Walraven GE, von Seidlein L. Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia. J Trop Pediatr. 2002;48(2):78-83.

129. O'Neil-Dunne I, Achur RN, Agbor-Enoh ST, Valiyaveettil M, Naik RS, Ockenhouse CF, et al. Gravidity-Dependent Production of Antibodies That Inhibit Binding of Plasmodium falciparum-Infected Erythrocytes to Placental Chondroitin Sulfate Proteoglycan during Pregnancy. Infect Immun. 2001;69(12):7487-92.

130. Walker PGT, Griffin JT, Cairns M, Rogerson SJ, van Eijk AM, ter Kuile F, et al. A model of parity-dependent immunity to placental malaria. Nat Commun. 2013;4:1609.

131. Moya-Alvarez V, Abellana R, Cot M. Pregnancy-associated malaria and malaria in infants: an old problem with present consequences. Malar J. 2014;13(1):1-10.

132. Recker M, Bouma MJ, Bamford P, Gupta S, Dobson AP. Assessing the burden of pregnancy-associated malaria under changing transmission settings. Malar J. 2009;8:245.

133. Nosten F, Rogerson SJ, Beeson JG, McGready R, Mutabingwa TK, Brabin B. Malaria in pregnancy and the endemicity spectrum: what can we learn? Trends Parasitol. 2004;20(9):425-32.

134. Greenwood BM. Immune responses to sporozoite antigens and their relationship to naturally acquired immunity to malaria. Bull World Health Organ. 1990;68 Suppl:184-90.

135. Steketee RW, Wirima JJ, Bloland PB, Chilima B, Mermin JH, Chitsulo L, et al. Impairment of a pregnant woman's acquired ability to limit Plasmodium falciparum by infection with human immunodeficiency virus type-1. Am J Trop Med Hyg. 1996;55(1 Suppl):42-9.

136. Newman RD, Hailemariam A, Jimma D, Degifie A, Kebede D, Rietveld AEC, et al. Burden of Malaria during Pregnancy in Areas of Stable and Unstable Transmission in Ethiopia during a Nonepidemic Year. J Infect Dis. 2003;187(11):1765-72.

137. Ndyomugyenyi R, Magnussen P. Malaria morbidity, mortality and pregnancy outcome in areas with different levels of malaria transmission in Uganda: a hospital record-based study. Trans R Soc Trop Med Hyg. 2001;95(5):463-8.

138. Boudova S, Cohee LM, Kalilani-Phiri L, Thesing PC, Kamiza S, Muehlenbachs A, et al. Pregnant women are a reservoir of malaria transmission in Blantyre, Malawi. Malar J. 2014;13:506.

139. Barnes KI, Little F, Mabuza A, Mngomezulu N, Govere J, Durrheim D, et al. Increased Gametocytemia after Treatment: An Early Parasitological Indicator of Emerging Sulfadoxine-Pyrimethamine Resistance in Falciparum Malaria. J Infect Dis. 2008;197(11):1605-13.

140. Fehintola FA, Balogun ST, Adeoye SB. Intermittent Preventive Treatment during Pregnancy with Sulphadoxine-Pyrimethamine May Promote Plasmodium falciparum Gametocytogenesis. Med Princ Pract. 2012;21(1):63-7.

141. WHO. Guidelines for the treatment of malaria. Second edition. Geneva: World Health Organization, 2010.

142. Bousema T, Okell L, Shekalaghe S, Griffin JT, Omar S, Sawa P, et al. Revisiting the circulation time of Plasmodium falciparum gametocytes: molecular detection methods to estimate the duration of gametocyte carriage and the effect of gametocytocidal drugs. Malar J. 2010;9:136.

143. Nevill CG, Some ES, Mung'ala VO, Mutemi W, New L, Marsh K, et al. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. Trop Med Int Health. 1996;1(2):139-46.

144. Premji Z, Lubega P, Hamisi Y, McHopa E, Minjas J, Checkley W, et al. Changes in malaria associated morbidity in children using insecticide treated mosquito nets in the Bagamoyo district of coastal Tanzania. Trop Med Parasitol. 1995;46(3):147-53.

145. Snow RW, Lindsay SW, Hayes RJ, Greenwood BM. Permethrin-treated bed nets (mosquito nets) prevent malaria in Gambian children. Trans R Soc Trop Med Hyg. 1988;82(6):838-42.

146. Müller O, Traoré C, Kouyaté B, Yé Y, Frey C, Coulibaly B, et al. Effects of insecticide-treated bednets during early infancy in an African area of intense malaria transmission: a randomized controlled trial. Bulletin of the World Health Organization. 2006;84(2):120-6.

147. Lengeler C. Insecticide-treated bed nets and curtains for preventing malaria. Cochrane Database Syst Rev. 2004(2):Cd000363.

148. Eisele TP, Larsen D, Steketee RW. Protective efficacy of interventions for preventing malaria mortality in children in Plasmodium falciparum endemic areas. Int J Epidemiol. 2010;39 Suppl 1:i88-101.

149. Thomson MC, D'Alessandro U, Bennett S, Connor SJ, Langerock P, Jawara M, et al. Malaria prevalence is inversely related to vector density in The Gambia, West Africa. Trans R Soc Trop Med Hyg. 1994;88(6):638-43.

150. Sexton JD, Ruebush TK, 2nd, Brandling-Bennett AD, Breman JG, Roberts JM, Odera JS, et al. Permethrin-impregnated curtains and bed-nets prevent malaria in western Kenya. Am J Trop Med Hyg. 1990;43(1):11-8.

151. Curtis CF, Jana-Kara B, Maxwell CA. Insecticide treated nets: impact on vector populations and relevance of initial intensity of transmission and pyrethroid resistance. J Vector Dis. 2003;40(1-2):1-8.

152. Kulkarni MA, Malima R, Mosha FW, Msangi S, Mrema E, Kabula B, et al. Efficacy of pyrethroid-treated nets against malaria vectors and nuisance-biting mosquitoes in Tanzania in areas with long-term insecticide-treated net use. Trop Med Int Health. 2007;12(9):1061-73.

153. Smithuis FM, Kyaw MK, Phe UO, van der Broek I, Katterman N, Rogers C, et al. The effect of insecticide-treated bed nets on the incidence and prevalence of malaria in children in an area of unstable seasonal transmission in western Myanmar. Malar J. 2013;12(1):1-15.

154. Coleman PG, Goodman CA, Mills A. Rebound mortality and the cost-effectiveness of malaria control: potential impact of increased mortality in late childhood following the introduction of insecticide treated nets. Trop Med Int Health. 1999;4(3):175-86.

155. Eisele TP, Lindblade KA, Wannemuehler KA, Gimnig JE, Odhiambo F, Hawley WA, et al. Effect of sustained insecticide-treated bed net use on all-cause child mortality in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg. 2005;73(1):149-56.

156. Louis VR, Bals J, Tiendrebeogo J, Bountogo M, Ramroth H, De Allegri M, et al. Long-term effects of malaria prevention with insecticide-treated mosquito nets on morbidity and mortality in African children: randomised controlled trial. Trop Med Int Health. 2012;17(6):733-41.

157. Shulman CE, Dorman EK, Talisuna AO, Lowe BS, Nevill C, Snow RW, et al. A community randomized controlled trial of insecticide-treated bednets for the prevention of malaria and anaemia among primigravid women on the Kenyan coast. Trop Med Int Health. 1998;3(3):197-204.

158. ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kariuki SK, et al. Reduction of malaria during pregnancy by permethrin-treated bed nets in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg. 2003;68(4 Suppl):50-60.

159. Gamble C, Ekwaru JP, ter Kuile FO. Insecticide-treated nets for preventing malaria in pregnancy. Cochrane Database Syst Rev. 2006(2):Cd003755.

160. Eisele TP, Larsen DA, Anglewicz PA, Keating J, Yukich J, Bennett A, et al. Malaria prevention in pregnancy, birthweight, and neonatal mortality: a meta-analysis of 32 national cross-sectional datasets in Africa. Lancet Infect Dis. 2012;12(12):942-9.

161. Gamble C, Ekwaru PJ, Garner P, ter Kuile FO. Insecticide-Treated Nets for the Prevention of Malaria in Pregnancy: A Systematic Review of Randomised Controlled Trials. PLoS Med. 2007;4(3):e107.

162. Lindblade KA, Mwandama D, Mzilahowa T, Steinhardt L, Gimnig J, Shah M, et al. A cohort study of the effectiveness of insecticide-treated bed nets to prevent malaria in an area of moderate pyrethroid resistance, Malawi. Malar J. 2015;14(1):1-15.

163. WHO. Insecticide-treated mosquito nets: a WHO position statement. Geneva: World Health Organization, 2007.

164. Feng G, Simpson JA, Chaluluka E, Molyneux ME, Rogerson SJ. Decreasing Burden of Malaria in Pregnancy in Malawian Women and Its Relationship to Use of Intermittent Preventive Therapy or Bed Nets. PLoS One. 2010;5(8):e12012.

165. WHO. WHO recommendations for achieving universal coverage with long-lasting insecticidal nets in malaria control. World Health Organization, 2013 2013. Report No.

166. Zarocostas J. Malaria treatment should begin with parasitological diagnosis where possible, says WHO. BMJ. 2010;340:c1402.

167. Reyburn H. New WHO guidelines for the treatment of malaria. BMJ. 2010;340:c2637.

168. Abba K, Kirkham AJ, Olliaro PL, Deeks JJ, Donegan S, Garner P, et al. Rapid diagnostic tests for diagnosing uncomplicated non-falciparum or Plasmodium vivax malaria in endemic countries. Cochrane Database Syst Rev. 2014;12:Cd011431.

169. Visser BJ, van Vugt M, Grobusch MP. Malaria: an update on current chemotherapy. Expert Opin Pharmacother. 2014;15(15):2219-54.

170. Visser BJ, Wieten RW, Kroon D, Nagel IM, Belard S, van Vugt M, et al. Efficacy and safety of artemisinin combination therapy (ACT) for non-falciparum malaria: a systematic review. Malar J. 2014;13:463.

171. Dellicour S, Hall S, Chandramohan D, Greenwood B. The safety of artemisinins during pregnancy: a pressing question. Malar J. 2007;6:15.

172. Nambozi M, Mulenga M, Halidou T, Tagbor H, Mwapasa V, Phiri LK, et al. Safe and efficacious artemisinin-based combination treatments for African pregnant women with malaria: a multicentre randomized control trial. Reprod Health. 2015;12:5.

173. Myint HY, Ashley EA, Day NP, Nosten F, White NJ. Efficacy and safety of dihydroartemisinin-piperaquine. Trans R Soc Trop Med Hyg. 2007;101(9):858-66.

174. Rijken MJ, McGready R, Boel ME, Barends M, Proux S, Pimanpanarak M, et al. Dihydroartemisinin-piperaquine rescue treatment of multidrug-resistant Plasmodium falciparum malaria in pregnancy: a preliminary report. Am J Trop Med Hyg. 2008;78(4):543-5.

175. Price Ric N DG, Nosten Francois. Antimalarial Therapies in Children from Papua New Guinea. N Engl J Mede. 2009;360(12):1254-5.

176. Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. Heart. 2003;89(11):1363-72.

177. White NJ. Cardiotoxicity of antimalarial drugs. Lancet Infect Dis. 2007;7(8):549-58.

178. Moore BR, Benjamin JM, Salman S, Griffin S, Ginny E, Page-Sharp M, et al. Effect of co-administered fat on the tolerability, safety and pharmacokinetic properties of dihydroartemisinin-piperaquine in Papua New Guinean children with uncomplicated malaria. Antimicrob Agents Chemother. 2014.

179. Karunajeewa H, Lim C, Hung T-Y, Ilett KF, Denis MB, Socheat D, et al. Safety evaluation of fixed combination piperaquine plus dihydroartemisinin (Artekin®) in Cambodian children and adults with malaria. Br J Clin Pharmacol. 2004;57(1):93-9.

180. Mytton OT, Ashley EA, Peto L, Price RN, La Y, Hae R, et al. Electrocardiographic Safety Evaluation of Dihydroartemisinin–Piperaquine in the Treatment of Uncomplicated falciparum Malaria. Am J Trop Med Hyg. 2007;77(3):447-50.

181. Lon C, Manning JE, Vanachayangkul P, So M, Sea D, Se Y, et al. Efficacy of Two versus Three-Day Regimens of Dihydroartemisinin-Piperaquine for Uncomplicated Malaria in Military Personnel in Northern Cambodia: An Open-Label Randomized Trial. PLoS One. 2014;9(3):e93138.

182. WHO. WHO policy brief for the implementation of intermittent preventive treatment of malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP) Geneva, Switzerland: World Health Organization; 2014 [cited 2015 22 March]. Available from: http://www.who.int/malaria/publications/atoz/policy_brief_iptp_sp_policy_recommendation/en/.

183. WHO. Guidelines on co-trimoxazole prophylaxis for HIV-related infections among children, adolescents and adults: recommendations for a public health approach. Geneva: 2006.

184. WHO. World Malaria Report 2013. Geneva, Switzerland: World Health Organization; 2013.

185. van Eijk AM, Hill J, Larsen DA, Webster J, Steketee RW, Eisele TP, et al. Coverage of intermittent preventive treatment and insecticide-treated nets for the control of malaria during pregnancy in sub-Saharan Africa: a synthesis and meta-analysis of national survey data, 2009-11. Lancet Infect Dis. 2013;13(12):1029-42.

186. National Malaria Control Programme (NMCP) II. Malawi Malaria Indicator Survey (MIS) 2012. Lilongwe, Malawi: 2012.

187. National Malaria Control Programme (NMCP) II. Malawi Malaria Indicator Survey (MIS) 2014. In: International NaI, editor. Lilongwe, Malawi and Calverton, Maryland2014.

188. Andrews KG, Lynch M, Eckert E, Gutman J. Missed opportunities to deliver intermittent preventive treatment for malaria to pregnant women 2003-2013: a systematic analysis of 58 household surveys in sub-Saharan Africa. Malar J. 2015;14(1):521.

189. ISRCTN registry. STOPMiP: Intermittent Screening and Treatment Or intermittent Preventive therapy for the control of Malaria in Pregnancy in Indonesia: BioMed Central; 2015 [cited 2015 27 April]. Available from: http://www.isrctn.com/ISRCTN34010937.

190. Plotkin M, Said K, Msellem MI, Chase RP, Hendler N, Khamis AR, et al. Placental Malaria Is Rare Among Zanzibari Pregnant Women Who Did Not Receive Intermittent Preventive Treatment in Pregnancy. Am J Trop Med Hyg. 2014;91(2):367-73.

191. Maiga OM, Kayentao K, Traoré BT, Djimde A, Traoré B, Diallo M, et al. Superiority of 3 Over 2 Doses of Intermittent Preventive Treatment With Sulfadoxine-Pyrimethamine for the Prevention of Malaria During Pregnancy in Mali: A Randomized Controlled Trial. Clin Infect Dis. 2011;53(3):215-23.

192. Mace KE, Chalwe V, Katalenich BL, Nambozi M, Mubikayi L, Mulele CK, et al. Evaluation of sulphadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnancy: a retrospective birth outcomes study in Mansa, Zambia. Malar J. 2015;14:69.

193. Mosha D, Chilongola J, Ndeserua R, Mwingira F, Genton B. Effectiveness of intermittent preventive treatment with sulfadoxine-pyrimethamine during pregnancy on placental malaria, maternal anaemia and birthweight in areas with high and low malaria transmission intensity in Tanzania. Trop Med Int Health. 2014;19(9):1048-56.

194. Filler SJ, Kazembe P, Thigpen M, Macheso A, Parise ME, Newman RD, et al. Randomized Trial of 2-Dose versus Monthly Sulfadoxine-Pyrimethamine Intermittent Preventive Treatment for Malaria in HIV-Positive and HIV-Negative Pregnant Women in Malawi. J Infect Dis. 2006;194(3):286-93.

195. Cot M, Le Hesran JY, Miailhes P, Esveld M, Etya'ale D, Breart G. Increase of birth weight following chloroquine chemoprophylaxis during the first pregnancy: results of a randomized trial in Cameroon. Am J Trop Med Hyg. 1995;53(6):581-5.

196. Wellems TE, Plowe CV. Chloroquine-Resistant Malaria. J Infect Dis. 2001;184(6):770-6.

197. Schultz LJ, Steketee RW, Macheso A, Kazembe P, Chitsulo L, Wirima JJ. The efficacy of antimalarial regimens containing sulfadoxine-pyrimethamine and/or chloroquine in preventing

peripheral and placental Plasmodium falciparum infection among pregnant women in Malawi. Am J Trop Med Hyg. 1994;51(5):515-22.

198. Shulman CE, Dorman EK, Cutts F, Kawuondo K, Bulmer JN, Peshu N, et al. Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. Lancet. 1999;353(9153):632-6.

199. Harrington WE, Mutabingwa TK, Muehlenbachs A, Sorensen B, Bolla MC, Fried M, et al. Competitive facilitation of drug-resistant Plasmodium falciparum malaria parasites in pregnant women who receive preventive treatment. Proc Natl Acad Sci U S A. 2009;106(22):9027-32.

200. Harrington WE, Mutabingwa TK, Kabyemela E, Fried M, Duffy PE. Intermittent treatment to prevent pregnancy malaria does not confer benefit in an area of widespread drug resistance. Clin Infect Dis. 2011;53(3):224-30.

201. Nosten F, White NJ. Artemisinin-based combination treatment of falciparum malaria. Am J Trop Med Hyg. 2007;77(6 Suppl):181-92.

202. Sibley CH, Hyde JE, Sims PF, Plowe CV, Kublin JG, Mberu EK, et al. Pyrimethamine-sulfadoxine resistance in Plasmodium falciparum: what next? Trends Parasitol. 2001;17(12):582-8.

203. Fohl LM, Roos DS. Fitness effects of DHFR-TS mutations associated with pyrimethamine resistance in apicomplexan parasites. Mol Microbiol. 2003;50(4):1319-27.

204. Mockenhaupt FP, Teun Bousema J, Eggelte TA, Schreiber J, Ehrhardt S, Wassilew N, et al. Plasmodium falciparum dhfr but not dhps mutations associated with sulphadoxine-pyrimethamine treatment failure and gametocyte carriage in northern Ghana. Trop Med Int Health. 2005;10(9):901-8.

205. Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria. J Infect Dis. 2002;185(3):380-8.

206. Tan KR, Katalenich BL, Mace KE, Nambozi M, Taylor SM, Meshnick SR, et al. Efficacy of sulphadoxine-pyrimethamine for intermittent preventive treatment of malaria in pregnancy, Mansa, Zambia. Malar J. 2014;13:227.

207. Taylor SM, Antonia AL, Chaluluka E, Mwapasa V, Feng G, Molyneux ME, et al. Antenatal Receipt of Sulfadoxine-Pyrimethamine Does Not Exacerbate Pregnancy-Associated Malaria Despite the Expansion of Drug-Resistant Plasmodium falciparum: Clinical Outcomes From the QuEERPAM Study. Clin Infect Dis. 2012;55(1):42-50.

208. Gesase S, Gosling RD, Hashim R, Ord R, Naidoo I, Madebe R, et al. High resistance of Plasmodium falciparum to sulphadoxine/pyrimethamine in northern Tanzania and the emergence of dhps resistance mutation at Codon 581. PLoS One. 2009;4(2):e4569.

209. Gutman J, Kalilani L, Taylor S, Zhou Z, Wiegand RE, Thwai KL, et al. The A581G Mutation in the Gene Encoding Plasmodium falciparum Dihydropteroate Synthetase Reduces the Effectiveness of Sulfadoxine-Pyrimethamine Preventive Therapy in Malawian Pregnant Women. The J Infect Dis. 2015.

210. Minja DTR, Schmiegelow C, Mmbando B, Boström S, Oesterholt M, Magistrado P, et al. Plasmodium falciparum Mutant Haplotype Infection during Pregnancy Associated with Reduced Birthweight, Tanzania. Emerg Infect Dis. 2013;19(9):1446-54.

211. Arinaitwe E, Ades V, Walakira A, Ninsiima B, Mugagga O, Patil TS, et al. Intermittent preventive therapy with sulfadoxine-pyrimethamine for malaria in pregnancy: a cross-sectional study from Tororo, Uganda. PLoS One. 2013;8(9):e73073.

212. WHO. WHO Policy recommendation on intermittent preventive treatment during infancy with sulphadoxine-pyrimethamine (IPTi-SP) for Plasmodium falciparum malaria control in Africa. Geneva, Switzerland: World Health Organization, 2010.

213. Sridaran S, McClintock SK, Syphard LM, Herman KM, Barnwell JW, Udhayakumar V. Anti-folate drug resistance in Africa: meta-analysis of reported dihydrofolate reductase (dhfr) and

dihydropteroate synthase (dhps) mutant genotype frequencies in African Plasmodium falciparum parasite populations. Malar J. 2010;9:247.

214. Venkatesan M, Alifrangis M, Roper C, Plowe CV. Monitoring antifolate resistance in intermittent preventive therapy for malaria. Trends Parasitol. 2013;29(10):10.1016/j.pt.2013.07.008.

215. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, et al. Reemergence of chloroquine-sensitive Plasmodium falciparum malaria after cessation of chloroquine use in Malawi. J Infect Dis. 2003;187(12):1870-5.

216. Mita T, Kaneko A, Lum JK, Bwijo B, Takechi M, Zungu IL, et al. Recovery of chloroquine sensitivity and low prevalence of the Plasmodium falciparum chloroquine resistance transporter gene mutation K76T following the discontinuance of chloroquine use in Malawi. Am J Trop Med Hyg. 2003;68(4):413-5.

217. Ndiaye M, Faye B, Tine R, Ndiaye JL, Lo A, Abiola A, et al. Assessment of the molecular marker of Plasmodium falciparum chloroquine resistance (Pfcrt) in Senegal after several years of chloroquine withdrawal. Am J Trop Med Hyg. 2012;87(4):640-5.

218. Artimovich E, Schneider K, Taylor TE, Kublin JG, Dzinjalamala FK, Escalante AA, et al. Persistence of Sulfadoxine-Pyrimethamine Resistance Despite Reduction of Drug Pressure in Malawi. J Infect Dis. 2015.

219. Iriemenam NC, Shah M, Gatei W, van Eijk AM, Ayisi J, Kariuki S, et al. Temporal trends of sulphadoxine-pyrimethamine (SP) drug-resistance molecular markers in Plasmodium falciparum parasites from pregnant women in western Kenya. Malar J. 2012;11:134.

220. Iyer JK, Milhous WK, Cortese JF, Kublin JG, Plowe CV. Plasmodium falciparum crossresistance between trimethoprim and pyrimethamine.Lancet. 2001;358(9287):1066-7.

221. Hastings IM, Watkins WM. Intensity of malaria transmission and the evolution of drug resistance. Acta Trop. 2005;94(3):218-29.

222. Mharakurwa S, Kumwenda T, Mkulama MA, Musapa M, Chishimba S, Shiff CJ, et al. Malaria antifolate resistance with contrasting Plasmodium falciparum dihydrofolate reductase (DHFR) polymorphisms in humans and Anopheles mosquitoes. Proc Natl Acad Sci U S A. 2011;108(46):18796-801.

223. Mharakurwa S, Sialumano M, Liu K, Scott A, Thuma P. Selection for chloroquine-sensitive Plasmodium falciparum by wild Anopheles arabiensis in Southern Zambia. Malar J. 2013;12:453.

224. Alifrangis M, Lemnge MM, Ronn AM, Segeja MD, Magesa SM, Khalil IF, et al. Increasing prevalence of wildtypes in the dihydrofolate reductase gene of Plasmodium falciparum in an area with high levels of sulfadoxine/pyrimethamine resistance after introduction of treated bed nets. Am J Trop Med Hyg. 2003;69(3):238-43.

225. Laufer MK, Takala-Harrison S, Dzinjalamala FK, Stine OC, Taylor TE, Plowe CV. Return of chloroquine-susceptible falciparum malaria in Malawi was a reexpansion of diverse susceptible parasites. J Infect Dis. 2010;202(5):801-8.

226. Nkhoma S, Molyneux M, Ward S. Molecular surveillance for drug-resistant Plasmodiumfalciparum malaria in Malawi. Acta Trop. 2007;102(2):138-42.

227. Njagi JK, Magnussen P, Estambale B, Ouma J, Mugo B. Prevention of anaemia in pregnancy using insecticide-treated bednets and sulfadoxine-pyrimethamine in a highly malarious area of Kenya: a randomized controlled trial. Trans R Soc Trop Med Hyg. 2003;97(3):277-82.

228. ClinicalTrials.gov. Chloroquine for Malaria in Pregnancy: U.S. National Institutes of Health; 2015 [cited 2015 27 April]. Available from: https://clinicaltrials.gov/ct2/show/NCT01443130.

229. Chico RM, Pittrof R, Greenwood B, Chandramohan D. Azithromycin-chloroquine and the intermittent preventive treatment of malaria in pregnancy. Malar J. 2008;7:255.

230. Chico RM, Chandramohan D. Azithromycin plus chloroquine: combination therapy for protection against malaria and sexually transmitted infections in pregnancy. EExpert Opin Drug Metab Toxicol. 2011;7(9):1153-67.

231. ClinicalTrials.gov. Evaluate Parasitological Clearance Rates And Pharmacokinetics Of The Combination Of Azithromycin And Chloroquine In Asymptomatic Pregnant Women With Falciparum Parasitemia In Africa: U.S. National Institutes of Health; 2015 [cited 2015 27 April]. Available from: https://clinicaltrials.gov/ct2/show/study/NCT01103713?term=NCT01103713&rank=1.

232. Luntamo M, Kulmala T, Mbewe B, Cheung YB, Maleta K, Ashorn P. Effect of Repeated Treatment of Pregnant Women with Sulfadoxine-Pyrimethamine and Azithromycin on Preterm Delivery in Malawi: A Randomized Controlled Trial. Am J Trop Med Hyg. 2010;83(6):1212-20.

233. Unger HW, Ome-Kaius M, Wangnapi RA, Umbers AJ, Hanieh S, Suen CS, et al. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. BMC Med. 2015;13:9.

234. Clerk CA, Bruce J, Affipunguh PK, Mensah N, Hodgson A, Greenwood B, et al. A randomized, controlled trial of intermittent preventive treatment with sulfadoxine-pyrimethamine, amodiaquine, or the combination in pregnant women in Ghana. J Infect Dis. 2008;198(8):1202-11.

235. Briand V, Bottero J, Noel H, Masse V, Cordel H, Guerra J, et al. Intermittent treatment for the prevention of malaria during pregnancy in Benin: a randomized, open-label equivalence trial comparing sulfadoxine-pyrimethamine with mefloquine. J Infect Dis. 2009;200(6):991-1001.

236. Gonzalez R, Mombo-Ngoma G, Ouedraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-negative women: a multicentre randomized controlled trial. PLoS Med. 2014;11(9):e1001733.

237. Gonzalez R, Desai M, Macete E, Ouma P, Kakolwa MA, Abdulla S, et al. Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-infected women receiving cotrimoxazole prophylaxis: a multicenter randomized placebo-controlled trial. PLoS Med. 2014;11(9):e1001735.

238. ClinicalTrials.gov. Efficacy of Intermittent Screening and Treatment or Intermittent Preventive Treatment (IPT) With Dihydroartemisinin-Piperaquine, Versus IPT With Sulfadoxine-Pyrimethamine for the Control of Malaria in Pregnancy in Kenya (STOP MiP KENYA): U.S. National

Institutes of Health; 2014 [cited 2015 27 April]. Available from: https://clinicaltrials.gov/ct2/show/NCT01669941.

239. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A Review of Malaria Diagnostic Tools: Microscopy and Rapid Diagnostic Test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119-27.

240. Coleman RE, Sattabongkot J, Promstaporm S, Maneechai N, Tippayachai B, Kengluecha A, et al. Comparison of PCR and microscopy for the detection of asymptomatic malaria in a Plasmodium falciparum/vivax endemic area in Thailand. Malar J. 2006;5:121.

241. Erdman LK, Kain KC. Molecular diagnostic and surveillance tools for global malaria control. Travel Med Infect Dis. 2008;6(1-2):82-99.

242. Rantala AM, Taylor SM, Trottman PA, Luntamo M, Mbewe B, Maleta K, et al. Comparison of real-time PCR and microscopy for malaria parasite detection in Malawian pregnant women. Malar J. 2010;9:269.

243. Cohee LM, Kalilani-Phiri L, Boudova S, Joshi S, Mukadam R, Seydel KB, et al. Submicroscopic malaria infection during pregnancy and the impact of intermittent preventive treatment. Malar J. 2014;13:274.

244. Doolan DL, Dobaño C, Baird JK. Acquired Immunity to Malaria. Clin Microbiol Rev. 2009;22(1):13-36.

245. Artavanis-Tsakonas K, Tongren JE, Riley EM. The war between the malaria parasite and the immune system: immunity, immunoregulation and immunopathology. Clin Exp Immunol. 2003;133(2):145-52.

246. Griffin JT, Hollingsworth TD, Reyburn H, Drakeley CJ, Riley EM, Ghani AC. Gradual acquisition of immunity to severe malaria with increasing exposure. Proc Biol Sci. 2015;282(1801).

247. Woodberry T, Minigo G, Piera KA, Amante FH, Pinzon-Charry A, Good MF, et al. Low-level Plasmodium falciparum blood-stage infection causes dendritic cell apoptosis and dysfunction in healthy volunteers. J Infect Dis. 2012;206(3):333-40.

248. Ibitokou SA, Boström S, Brutus L, Tuikue Ndam N, Vianou B, Agbowaï C, et al. Submicroscopic Infections with Plasmodium falciparum during Pregnancy and Their Association with Circulating Cytokine, Chemokine, and Cellular Profiles. Clin Vaccine Immunol. 2014;21(6):859-66.

249. Wilson NO, Bythwood T, Solomon W, Jolly P, Yatich N, Jiang Y, et al. Elevated Levels of IL-10 and G-CSF Associated with Asymptomatic Malaria in Pregnant Women. Infect Dis Obstet Gynecol. 2010;2010:7.

250. Hugosson E, Montgomery SM, Premji Z, Troye-Blomberg M, Björkman A. Higher IL-10 levels are associated with less effective clearance of Plasmodium falciparum parasites. Parasite Immunol. 2004;26(3):111-7.

251. Othoro C, Lal AA, Nahlen B, Koech D, Orago ASS, Udhayakumar V. A Low Interleukin-10 Tumor Necrosis Factor-α Ratio Is Associated with Malaria Anemia in Children Residing in a Holoendemic Malaria Region in Western Kenya. J Infect Dis. 1999;179(1):279-82.

252. Agudelo OM, Aristizabal BH, Yanow SK, Arango E, Carmona-Fonseca J, Maestre A. Submicroscopic infection of placenta by Plasmodium produces Th1/Th2 cytokine imbalance, inflammation and hypoxia in women from north-west Colombia. Malar J. 2014;13:122.

253. Singh N, Bharti PK, Singh MP, Singh R, Yeboah-Antwi K, Desai M, et al. What is the burden of submicroscopic malaria in pregnancy in central India? Pathog Glob Health. 2015;109(1):30-8.

254. Walker-Abbey A, Djokam RR, Eno A, Leke RF, Titanji VP, Fogako J, et al. Malaria in pregnant Cameroonian women: the effect of age and gravidity on submicroscopic and mixed-species infections and multiple parasite genotypes. Am J Trop Med Hyg. 2005;72(3):229-35.

255. Cottrell G, Moussiliou A, Luty AJ, Cot M, Fievet N, Massougbodji A, et al. Submicroscopic Plasmodium falciparum Infections Are Associated With Maternal Anemia, Premature Births, and Low Birth Weight. Clin Infect Dis. 2015.

256. Mockenhaupt FP, Rong B, Till H, Eggelte TA, Beck S, Gyasi-Sarpong C, et al. Submicroscopic Plasmodium falciparum infections in pregnancy in Ghana. Trop Med Int Health. 2000;5(3):167-73.

257. Arango EM, Samuel R, Agudelo OM, Carmona-Fonseca J, Maestre A, Yanow SK. Molecular Detection of Malaria at Delivery Reveals a High Frequency of Submicroscopic Infections and Associated Placental Damage in Pregnant Women from Northwest Colombia. Am J Trop Med Hyg. 2013;89(1):178-83.

258. Mutanda AL, Cheruiyot P, Hodges JS, Ayodo G, Odero W, John CC. Sensitivity of fever for diagnosis of clinical malaria in a Kenyan area of unstable, low malaria transmission. Malar J. 2014;13:163.

259. Muhe L, Oljira B, Degefu H, Enquesellassie F, Weber MW. Clinical algorithm for malaria during low and high transmission seasons. Arch Dis Child. 1999;81(3):216-20.

260. Kallander K, Nsungwa-Sabiiti J, Peterson S. Symptom overlap for malaria and pneumonia--policy implications for home management strategies. Acta Trop. 2004;90(2):211-4.

261. Reyburn H, Mbatia R, Drakeley C, Carneiro I, Mwakasungula E, Mwerinde O, et al. Overdiagnosis of malaria in patients with severe febrile illness in Tanzania: a prospective study. BMJ. 2004;329(7476):1212.

262. Jelinek T, Grobusch MP, Schwenke S, Steidl S, von Sonnenburg F, Nothdurft HD, et al. Sensitivity and specificity of dipstick tests for rapid diagnosis of malaria in nonimmune travelers. J Clin Microbiol. 1999;37(3):721-3.

263. Gerstl S, Dunkley S, Mukhtar A, De Smet M, Baker S, Maikere J. Assessment of two malaria rapid diagnostic tests in children under five years of age, with follow-up of false-positive pLDH test results, in a hyperendemic falciparum malaria area, Sierra Leone. Malar J. 2010;9:28.

264. Endeshaw T, Gebre T, Ngondi J, Graves PM, Shargie EB, Ejigsemahu Y, et al. Evaluation of light microscopy and rapid diagnostic test for the detection of malaria under operational field conditions: a household survey in Ethiopia. Malar J. 2008;7:118.

265. Bejon P, Andrews L, Hunt-Cooke A, Sanderson F, Gilbert SC, Hill AVS. Thick blood film examination for Plasmodium falciparum malaria has reduced sensitivity and underestimates parasite density. Malar J. 2006;5:104-.

266. CDC. Malaria Diagnosis (U.S.) - Microscopy Atlanta, USA: Centers for Disease Control and Prevention; 2012 [updated 9 November 2012; cited 2015 28 March]. Available from: http://www.cdc.gov/malaria/diagnosis_treatment/diagnosis.html.

267. McKenzie FE, Sirichaisinthop J, Miller RS, Gasser RA, Jr., Wongsrichanalai C. Dependence of malaria detection and species diagnosis by microscopy on parasite density. Am J Trop Med Hyg. 2003;69(4):372-6.

268. Maguire JD, Lederman ER, Barcus MJ, O'Meara WA, Jordon RG, Duong S, et al. Production and validation of durable, high quality standardized malaria microscopy slides for teaching, testing and quality assurance during an era of declining diagnostic proficiency. Malar J. 2006;5:92.

269. Johnston SP, Pieniazek NJ, Xayavong MV, Slemenda SB, Wilkins PP, da Silva AJ. PCR as a confirmatory technique for laboratory diagnosis of malaria. J Clin Microbiol. 2006;44(3):1087-9.

270. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet. 2004;363(9414):1017-24.

271. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. Plasmodium knowlesi malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46(2):165-71.

272. Clendennen TE, 3rd, Long GW, Baird JK. QBC and Giemsa-stained thick blood films: diagnostic performance of laboratory technologists. Trans R Soc Trop Med Hyg. 1995;89(2):183-4.

273. Lenz D, Kremsner PG, Lell B, Biallas B, Boettcher M, Mordmuller B, et al. Assessment of LED fluorescence microscopy for the diagnosis of Plasmodium falciparum infections in Gabon. Malar J. 2011;10:194.

274. Ochola LB, Vounatsou P, Smith T, Mabaso MLH, Newton C. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. Lancet Infect Dis. 2006;6(9):582-8.

275. She RC, Rawlins ML, Mohl R, Perkins SL, Hill HR, Litwin CM. Comparison of immunofluorescence antibody testing and two enzyme immunoassays in the serologic diagnosis of malaria. J Travel Med. 2007;14(2):105-11.

276. Duo-Quan W, Lin-Hua T, Zhen-Cheng G, Xiang Z, Man-Ni Y. Application of the indirect fluorescent antibody assay in the study of malaria infection in the Yangtze River Three Gorges Reservoir, China. Malar J. 2009;8:199.

277. Noedl H, Yingyuen K, Laoboonchai A, Fukuda M, Sirichaisinthop J, Miller RS. Sensitivity and specificity of an antigen detection ELISA for malaria diagnosis. Am J Trop Med Hyg. 2006;75(6):1205-8.

278. WHO. Malaria rapid diagnostic test performance: results of WHO product testing of malaria RDTs: round 1 (2008). Geneva, Switzerland: World Health Organization, 2008.

279. Bell D, Peeling RW. Evaluation of rapid diagnostic tests: malaria. Nat Rev Micro. 2006.

280. WHO. Malaria Rapid Diagnostic Tests: Accuracy of results: World Health Organization; 2005 [cited 2015 26 March]. Available from: http://www.wpro.who.int/malaria/sites/rdt/whatis/action.html.

281. Hopkins H, Bebell L, Kambale W, Dokomajilar C, Rosenthal PJ, Dorsey G. Rapid diagnostic tests for malaria at sites of varying transmission intensity in Uganda. J Infect Dis. 2008;197(4):510-8.

282. Abeku TA, Kristan M, Jones C, Beard J, Mueller DH, Okia M, et al. Determinants of the accuracy of rapid diagnostic tests in malaria case management: evidence from low and moderate transmission settings in the East African highlands. Malar J. 2008;7:202.

283. Minja DT, Schmiegelow C, Oesterholt M, Magistrado PA, Bostrom S, John D, et al. Reliability of rapid diagnostic tests in diagnosing pregnancy-associated malaria in north-eastern Tanzania. Malar J. 2012;11:211.

284. Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, et al. A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS One. 2010;5(1):e8091.

285. Maltha J, Gamboa D, Bendezu J, Sanchez L, Cnops L, Gillet P, et al. Rapid diagnostic tests for malaria diagnosis in the Peruvian Amazon: impact of pfhrp2 gene deletions and cross-reactions. PLoS One. 2012;7(8):e43094.

286. Kumar N, Pande V, Bhatt RM, Shah NK, Mishra N, Srivastava B, et al. Genetic deletion of HRP2 and HRP3 in Indian Plasmodium falciparum population and false negative malaria rapid diagnostic test. Acta Trop. 2013;125(1):119-21.

287. Wurtz N, Fall B, Bui K, Pascual A, Fall M, Camara C, et al. Pfhrp2 and pfhrp3 polymorphisms in Plasmodium falciparum isolates from Dakar, Senegal: impact on rapid malaria diagnostic tests. Malar J. 2013;12:34.

288. Ho MF, Baker J, Lee N, Luchavez J, Ariey F, Nhem S, et al. Circulating antibodies against Plasmodium falciparum histidine-rich proteins 2 interfere with antigen detection by rapid diagnostic tests. Malar J. 2014;13:480.

289. Iqbal J, Sher A, Rab A. Plasmodium falciparum histidine-rich protein 2-based immunocapture diagnostic assay for malaria: cross-reactivity with rheumatoid factors. J Clin Microbiol. 2000;38(3):1184-6.

290. Mtove G, Hendriksen IC, Amos B, Mrema H, Mandia V, Manjurano A, et al. Treatment guided by rapid diagnostic tests for malaria in Tanzanian children: safety and alternative bacterial diagnoses. Malar J. 2011;10:290.

291. Meatherall B, Preston K, Pillai DR. False positive malaria rapid diagnostic test in returning traveler with typhoid fever. BMC Infect Dis. 2014;14:377.

292. CDC. Malaria Diagnosis (U.S.) – Rapid Diagnostic Test Atlanta, USA: Centers for Disease Control and Prevention; 2014 [updated 14 July 2014; cited 2015 28 March]. Available from: http://www.cdc.gov/malaria/diagnosis_treatment/rdt.html.

293. Mouatcho JC, Goldring JPD. Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol. 2013;62(Pt 10):1491-505.

294. Tanizaki R, Kato Y, Iwagami M, Kutsuna S, Ujiie M, Takeshita N, et al. Performance of Rapid Diagnostic Tests for Plasmodium ovale Malaria in Japanese Travellers. Trop Med Health. 2014;42(4):149-53.

295. Foster D, Cox-Singh J, Mohamad DS, Krishna S, Chin PP, Singh B. Evaluation of three rapid diagnostic tests for the detection of human infections with Plasmodium knowlesi. Malar J. 2014;13:60.

296. Kantele A, Jokiranta TS. Review of Cases With the Emerging Fifth Human Malaria Parasite, Plasmodium knowlesi. Clin Infect Dis. 2011;52(11):1356-62.

297. Jeremiah SS, Janagond AB, Parija SC. Challenges in diagnosis of Plasmodium knowlesi infections. Trop Parasitol. 2014;4(1):25-30.

298. Maltha J, Gillet P, Jacobs J. Malaria rapid diagnostic tests in endemic settings. Clin Microbiol Infect. 2013;19(5):399-407.

299. WHO. Summary results of WHO product testing of malaria RDTs: Round 1-5 (2008-2013). Geneva, Switzerland: World Health Organization, 2013.

300. UNITAID. Malaria Diagnostics Technology and Market Landscape. 2nd Edition. Geneva, Switzerland: World Health Organization, 2014.

301. Schachterle SE, Mtove G, Levens JP, Clemens EG, Shi L, Raj A, et al. Prevalence and Density-Related Concordance of Three Diagnostic Tests for Malaria in a Region of Tanzania with Hypoendemic Malaria. J Clin Microbiol. 2011;49(11):3885-91.

302. Wangai LN, Karau MG, Njiruh PN, Sabah O, Kimani FT, Magoma G, et al. Sensitivity of Microscopy Compared to Molecular Diagnosis of P. Falciparum: Implications on Malaria Treatment in Epidemic Areas in Kenya. African J Infect Dis. 2011;5(1):1-6.

303. Fransisca L, Kusnanto JH, Satoto TBT, Sebayang B, Supriyanto , Andriyan E, et al. Comparison of rapid diagnostic test Plasmotec Malaria-3, microscopy, and quantitative real-time PCR for diagnoses of Plasmodium falciparum and Plasmodium vivax infections in Mimika Regency, Papua, Indonesia. Malar J. 2015;14:103.

304. Snounou G, Viriyakosol S, Jarra W, Thaithong S, Brown KN. Identification of the four human malaria parasite species in field samples by the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitol. 1993;58(2):283-92.

305. Lucchi NW, Poorak M, Oberstaller J, DeBarry J, Srinivasamoorthy G, Goldman I, et al. A new single-step PCR assay for the detection of the zoonotic malaria parasite Plasmodium knowlesi. PLoS One. 2012;7(2):e31848.

306. Link L, Bart A, Verhaar N, van Gool T, Pronk M, Scharnhorst V. Molecular Detection of Plasmodium knowlesi in a Dutch Traveler by Real-Time PCR. J Clin Microbiol. 2012;50(7):2523-4.

307. Bosman P, Stassijns J, Nackers F, Canier L, Kim N, Khim S, et al. Plasmodium prevalence and artemisinin-resistant falciparum malaria in Preah Vihear Province, Cambodia: a cross-sectional population-based study. Malar J. 2014;13:394.

308. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois A-C, Khim N, et al. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014;505(7481):50-5.

309. Alker AP, Mwapasa V, Meshnick SR. Rapid real-time PCR genotyping of mutations associated with sulfadoxine-pyrimethamine resistance in Plasmodium falciparum. Antimicrob Agents Chemother. 2004;48(8):2924-9.

310. Mishra S, Raj DK, Hazra RK, Dash AP, Supakar PC. An efficient PCR--SSCP-based method for detection of a chloroquine resistance marker in the PfCRT gene of Plasmodium falciparum. Trans R Soc Trop Med Hyg. 2006;100(3):243-7.

311. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria diagnosis: a brief review. Korean J Parasitol. 2009;47(2):93-102.

312. Chaikitgosiyakul S, Rijken MJ, Muehlenbachs A, Lee SJ, Chaisri U, Viriyavejakul P, et al. A morphometric and histological study of placental malaria shows significant changes to villous architecture in both Plasmodium falciparum and Plasmodium vivax infection. Malar J. 2014;13:4.

313. Eusebi P. Diagnostic accuracy measures. Cerebrovasc Dis. 2013;36(4):267-72.

314. Parikh R, Mathai A, Parikh S, Chandra Sekhar G, Thomas R. Understanding and using sensitivity, specificity and predictive values. Indian J Ophthalmol. 2008;56(1):45-50.

315. Harper R, Reeves B. Reporting of precision of estimates for diagnostic accuracy: a review. BMJ. 1999;318(7194):1322-3.

316. Deeks JJ, Altman DG. Sensitivity and specificity and their confidence intervals cannot exceed 100%. BMJ. 1999;318(7177):193-4.

317. Shiu S-Y, Gatsonis C. The predictive receiver operating characteristic curve for the joint assessment of the positive and negative predictive values. Philosophical Transactions of the Royal Society of London A: Mathematical, Physical and Engineering Sciences. 2008;366(1874):2313-33.

318. Coulthard MG. Quantifying how tests reduce diagnostic uncertainty. Arch Dis Child. 2007;92(5):404-8.

319. BMJ. Diagnostic test studies: assessment and critical appraisal: BMJ Publishing; 2014 [cited 2015 30 March]. Available from: http://clinicalevidence.bmj.com/x/set/static/ebm/toolbox/665061.html.

320. Šimundić A-M. Measures of diagnostic accuracy: basic definitions. Med Biol Sci. 2008;22(4):61-5.

321. Bossuyt PM, Reitsma JB, Bruns DE, Gatsonis CA, Glasziou PP, Irwig LM, et al. The STARD statement for reporting studies of diagnostic accuracy: explanation and elaboration. Ann Intern Med. 2003;138(1):W1-12.

322. Bossuyt PMM. STARD Statement: Still Room for Improvement in the Reporting of Diagnostic Accuracy Studies. Radiology. 2008;248(3):713-4.

323. Sheehy AM, Coursin DB, Gabbay RA. Back to Wilson and Jungner: 10 Good Reasons to Screen for Type 2 Diabetes Mellitus. Mayo Clin Proc. 2009;84(1):38-42.

324. Harris R, Sawaya GF, Moyer VA, Calonge N. Reconsidering the Criteria for Evaluating Proposed Screening Programs: Reflections From 4 Current and Former Members of the U.S. Preventive Services Task Force. Epidemiol Rev. 2011;33(1):20-35.

325. Forman J, Coyle F, Levy-Fisch J, Roberts P, Terry S, Legge M. Screening criteria: the need to deal with new developments and ethical issues in newborn metabolic screening. J Community Genet. 2013;4(1):59-67.

326. Andermann A, Blancquaert I, Beauchamp S, Dery V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. Bull World Health Organ. 2008;86(4):317-9.

327. Petros M. Revisiting the Wilson-Jungner criteria: how can supplemental criteria guide public health in the era of genetic screening? Genet Med. 2012;14(1):129-34.

328. WHO. Delivering HIV test results and messages for re-testing and counselling in adults. Geneva: World Health Organization, 2010.

329. Tako EA, Zhou A, Lohoue J, Leke R, Taylor DW, Leke RF. Risk factors for placental malaria and its effect on pregnancy outcome in Yaounde, Cameroon. Am J Trop Med Hyg. 2005;72(3):236-42.

330. Katz J, Lee AC, Kozuki N, Lawn JE, Cousens S, Blencowe H, et al. Mortality risk in preterm and small-for-gestational-age infants in low-income and middle-income countries: a pooled country analysis. Lancet. 2013;382(9890):417-25.

331. Bennett A, Kazembe L, Mathanga DP, Kinyoki D, Ali D, Snow RW, et al. Mapping Malaria Transmission Intensity in Malawi, 2000–2010. Am J Trop Med Hyg. 2013;89(5):840-9.

332. Malawi. Ministry of Health. Guidelines for paediatric HIV testing and counselling, Malawi. Lilongwe: Ministry of Health; 2007. vi, 25 p. p.

333. CPMP. Points to consider on switching between superiority and non-inferiority (Committee for Proprietory Medicinal Products). Br J Clin Psychol. 2001;52:223-8.

334. Noel-Weiss J, Courant G, Woodend AK. Physiological weight loss in the breastfed neonate: a systematic review. Open Med. 2008;2(4):e99-e110.

335. Flaherman VJ, Kuzniewicz MW, Li S, Walsh E, McCulloch CE, Newman TB. First-day weight loss predicts eventual weight nadir for breastfeeding newborns. Arch Dis Child Fetal Neonatal Ed. 2013;98(6):F488-92.

336. Greenwood BM, Greenwood AM, Snow RW, Byass P, Bennett S, Hatib-N'Jie AB. The effects of malaria chemoprophylaxis given by traditional birth attendants on the course and outcome of pregnancy. Trans R Soc Trop Med Hyg. 1989;83(5):589-94.

337. D'Alessandro U, Langerock P, Bennett S, Francis N, Cham K, Greenwood BM. The impact of a national impregnated bed net programme on the outcome of pregnancy in primigravidae in The Gambia. Trans R Soc Trop Med Hyg. 1996;90(5):487-92.

338. Tagbor H, Bruce J, Agbo M, Greenwood B, Chandramohan D. Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: a randomised controlled non-inferiority trial. PLoS One. 2010;5(12):e14425.

339. Tagbor H, Cairns M, Bojang K, Coulibaly SO, Kayentao K, Williams JE, et al. A non-inferiority, individually randomised trial of intermittent screening and treatment: an alternative approach to the control of malaria in pregnancy. PLoS One. 2015 (in press).

340. Artimovich E, Schneider K, Taylor TE, Kublin JG, Dzinjalamala FK, Escalante AA, et al. Persistence of sulfadoxine-pyrimethamine resistance despite reduction of drug pressure in Malawi. J Infect Dis. 2015.

341. Naidoo I, Roper C. Mapping 'partially resistant', 'fully resistant', and 'super resistant' malaria. Trends Parasitol. 2013;29(10):505-15.

342. ter Kuile FO, et al. Impact of sulphadoxine-pyrimethamine resistance on the effectiveness of Intermittent Preventive Therapy for malaria in pregnancy (IPTp) in Africa: A systematic review and meta-analysis. Report for WHO ERG. 2013.

343. Desai M, Gutman J, Taylor S, Wiegand R, Khairallah C, Kayentao K, et al. Impact of sulfadoxine-pyrimethamine resistance on effectiveness of intermittent preventive therapy in pregnancy at clearing peripheral blood plasmodium falciparum parasites and preventing low birth weight: A prospective, multi-country observational study across Africa. Submitted.

344. Capan M, Mombo-Ngoma G, Makristathis A, Ramharter M. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin. Malar J. 2010;9:303.

345. Cook J, Xu W, Msellem M, Vonk M, Bergstrom B, Gosling R, et al. Mass screening and treatment on the basis of results of a Plasmodium falciparum-specific rapid diagnostic test did not reduce malaria incidence in Zanzibar. J Infect Dis. 2015;211(9):1476-83.

346. von Seidlein L. The Failure of Screening and Treating as a Malaria Elimination Strategy. PLoS Med. 2014;11(1):e1001595.

347. White NJ. How antimalarial drug resistance affects post-treatment prophylaxis. Malar J. 2008;7:9-.

348. Dorsey G, Gasasira AF, Machekano R, Kamya MR, Staedke SG, Hubbard A. The impact of age, temperature, and parasite density on treatment outcomes from antimalarial clinical trials in Kampala, Uganda. Am J Trop Med Hyg. 2004;71(5):531-6.

349. Vallely A, Vallely L, Changalucha J, Greenwood B, Chandramohan D. Intermittent preventive treatment for malaria in pregnancy in Africa: what's new, what's needed? Malar J. 2007;6:16.

350. Rijken MJ, McGready R, Phyo AP, Lindegardh N, Tarning J, Laochan N, et al. Pharmacokinetics of Dihydroartemisinin and Piperaquine in Pregnant and Nonpregnant Women with Uncomplicated Falciparum Malaria. Antimicrob Agents Chemother. 2011;55(12):5500-6.

351. The Pregnancy and Artemisinin-based Combination Treatments (PREGACT) study group, Tinto H, Traoré/Coulibaly M, Tahita MC, Valéa I, Mwapasa V, et al. The safety and efficacy of four artemisinin-based combination treatments in African pregnant women with malaria [submitted].

352. Pell C, Menaca A, Chatio S, Hodgson A, Tagbor H, Pool R. The acceptability of intermittent screening and treatment versus intermittent preventive treatment during pregnancy: results from a qualitative study in Northern Ghana. Malar J. 2014;13:432.

353. Smith Paintain L, Antwi GD, Jones C, Amoako E, Adjei RO, Afrah NA, et al. Intermittent screening and treatment versus intermittent preventive treatment of malaria in pregnancy: provider knowledge and acceptability. PLoS One. 2011;6(8):e24035.

354. Zou G. A Modified Poisson Regression Approach to Prospective Studies with Binary Data. Am J Epidemiol. 2004;159(7):702-6.

355. Cummings P. Methods for estimating adjusted risk ratios. Stata Journal. 2009;9(2):175-96.

356. Cameron AC TP. Microeconometrics Using Stata. College Station (TX): Stata Press; 2009.

357. Brabin BJ. An analysis of malaria in pregnancy in Africa. Bull World Health Organ. 1983;61(6):1005-16.

358. Tutterrow YL, Salanti A, Avril M, Smith JD, Pagano IS, Ako S, et al. High Avidity Antibodies to Full-Length VAR2CSA Correlate with Absence of Placental Malaria. PLoS One. 2012;7(6):e40049.

359. Ndam NT, Denoeud-Ndam L, Doritchamou J, Viwami F, Salanti A, Nielsen MA, et al. Protective Antibodies against Placental Malaria and Poor Outcomes during Pregnancy, Benin. Emerg Infect Dis. 2015;21(5):813-23.

360. Ter Kuile FO, Terlouw DJ, Phillips-Howard PA, Hawley WA, Friedman JF, Kariuki SK, et al. Reduction of malaria during pregnancy by Perimethrin-treated bed nets in an area of intense perennial malaria transmission in western Kenya. Am J Trop Med Hyg. 2003;68(4 suppl):50-60.

361. Gamble C, Ekwaru PJ, Garner P, ter Kuile FO. Insecticide-Treated Nets for the Prevention of Malaria in Pregnancy: A Systematic Review of Randomised Controlled Trials. PLoS Med. 2007;4(3):e107.

362. Mouatcho JC, Goldring JP. Malaria rapid diagnostic tests: challenges and prospects. J Med Microbiol. 2013;62(Pt 10):1491-505.

363. Moody A. Rapid Diagnostic Tests for Malaria Parasites. Clin Microbiol Rev. 2002;15(1):66-78.

364. McMorrow ML, Aidoo M, Kachur SP. Malaria rapid diagnostic tests in elimination settings—can they find the last parasite? Clin Microbiol Infect. 2011;17(11):1624-31.

365. Matangila JR, Lufuluabo J, Ibalanky AL, Inocencio da Luz RA, Lutumba P, Van Geertruyden JP. Asymptomatic Plasmodium falciparum infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of the Congo. Malar J. 2014;13:132.

366. Ndyomugyenyi R, Magnussen P. Anaemia in pregnancy: Plasmodium falciparum infection is an important cause in primigravidae in Hoima district, western Uganda. Ann Trop Med Parasitol. 1999;93(5):457-65.

367. Malhotra I, Dent A, Mungai P, Muchiri E, King CL. Real-Time Quantitative PCR for Determining the Burden of Plasmodium falciparum Parasites during Pregnancy and Infancy. J Clin Microbiol. 2005;43(8):3630-5.

368. Ayoola OO, Gemmell I, Omotade OO, Adeyanju OA, Cruickshank JK, Clayton PE. Maternal Malaria, Birth Size and Blood Pressure in Nigerian Newborns: Insights into the Developmental Origins of Hypertension from the Ibadan Growth Cohort. PLoS One. 2011;6(9):e24548.

369. Kalilani L, Mofolo I, Chaponda M, Rogerson SJ, Meshnick SR. The effect of timing and frequency of Plasmodium falciparum infection during pregnancy on the risk of low birth weight and maternal anemia. Trans R Soc Trop Med Hyg. 2010;104(6):416-22.

370. Valea I, Tinto H, Drabo MK, Huybregts L, Sorgho H, Ouedraogo JB, et al. An analysis of timing and frequency of malaria infection during pregnancy in relation to the risk of low birth weight, anaemia and perinatal mortality in Burkina Faso. Malar J. 2012;11:71.

371. Cottrell G, Mary J-Y, Barro D, Cot M. The importance of the period of malarial infection during pregnancy on birth weight in tropical Africa. Am J Trop Med Hyg. 2007;76(5):849-54.

372. Takem EN, Roca A, Cunnington A. The association between malaria and non-typhoid Salmonella bacteraemia in children in sub-Saharan Africa: a literature review. Malar J. 2014;13:400.

373. Church J, Maitland K. Invasive bacterial co-infection in African children with Plasmodium falciparum malaria: a systematic review. BMC Med. 2014;12:31.

374. Maltha J, Guiraud I, Kaboré B, Lompo P, Ley B, Bottieau E, et al. Frequency of Severe Malaria and Invasive Bacterial Infections among Children Admitted to a Rural Hospital in Burkina Faso. PLoS One. 2014;9(2):e89103.

375. Brabin BJ, Romagosa C, Abdelgalil S, Menendez C, Verhoeff FH, McGready R, et al. The sick placenta-the role of malaria. Placenta. 2004;25(5):359-78.

376. Stanisic DI, Moore KA, Baiwog F, Ura A, Clapham C, King CL, et al. Risk factors for malaria and adverse birth outcomes in a prospective cohort of pregnant women resident in a high malaria transmission area of Papua New Guinea. Trans R Soc Trop Med Hyg. 2015.

377. Takem EN, D'Alessandro U. Malaria in Pregnancy. Mediterranean Journal of Hematology and Infectious Diseases. 2013;5(1):e2013010.

378. Douamba Z, Bisseye C, Djigma FW, Compaor, #xe9, TR, et al. Asymptomatic Malaria Correlates with Anaemia in Pregnant Women at Ouagadougou, Burkina Faso. J Biomed Biotechnol. 2012;2012:6.

379. Anchang-Kimbi JK, Achidi EA, Nkegoum B, Sverremark-Ekstrom E, Troye-Blomberg M. Diagnostic comparison of malaria infection in peripheral blood, placental blood and placental biopsies in Cameroonian parturient women. Malar J. 2009;8:126.

380. Leke RF, Djokam RR, Mbu R, Leke RJ, Fogako J, Megnekou R, et al. Detection of the Plasmodium falciparum antigen histidine-rich protein 2 in blood of pregnant women: implications for diagnosing placental malaria. J Clin Microbiol. 1999;37(9):2992-6.

381. Mayor A, Moro L, Aguilar R, Bardaji A, Cistero P, Serra-Casas E, et al. How hidden can malaria be in pregnant women? Diagnosis by microscopy, placental histology, polymerase chain reaction and detection of histidine-rich protein 2 in plasma. Clin Infect Dis. 2012;54(11):1561-8.

382. Kattenberg JH, Ochodo EA, Boer KR, Schallig HD, Mens PF, Leeflang MM. Systematic review and meta-analysis: rapid diagnostic tests versus placental histology, microscopy and PCR for malaria in pregnant women. Malar J. 2011;10:321.

383. Tamhane A. Matched-Sample Analysis: Sensitivity, Specificity, and P Values. Clin Infect Dis. 2008;46(3):474-5.

384. Kim S, Lee W. Does McNemar's test compare the sensitivities and specificities of two diagnostic tests? Stat Methods Med Res. 2014.

385. Alfred N, Lovette L, Aliyu G, Olusegun O, Meshak P, Jilang T, et al. Optimising Mycobacterium tuberculosis detection in resource limited settings. BMJ Open. 2014;4(3).

386. Njama-Meya D, Kamya MR, Dorsey G. Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children. Trop Med Int Health. 2004;9(8):862-8.

387. Ilombe G, Maketa V, Mavoko HM, da Luz RI, Lutumba P, Van geertruyden JP. Performance of HRP2-based rapid test in children attending the health centre compared to asymptomatic children in the community. Malar J. 2014;13:308.

388. Bharti PK, Silawat N, Singh PP, Singh MP, Shukla M, Chand G, et al. The usefulness of a new rapid diagnostic test, the First Response Malaria Combo (pLDH/HRP2) card test, for malaria diagnosis in the forested belt of central India. Malar J. 2008;7:126.

389. Hendriksen ICE, Mtove G, Pedro AJ, Gomes E, Silamut K, Lee SJ, et al. Evaluation of a PfHRP2 and a pLDH-based Rapid Diagnostic Test for the Diagnosis of Severe Malaria in 2 Populations of African Children. Clin Infect Dis. 2011;52(9):1100-7.

390. Fried M, Muehlenbachs A, Duffy PE. Diagnosing malaria in pregnancy: an update. Expert review of anti-infective therapy. 2012;10(10):1177-87.

391. Kashif AH, Adam GK, Mohmmed AA, Elzaki SE, AbdelHalim AM, Adam I. Reliability of rapid diagnostic test for diagnosing peripheral and placental malaria in an area of unstable malaria transmission in Eastern Sudan. Diagn Pathol. 2013;8:59.

392. Kyabayinze DJ, Tibenderana JK, Nassali M, Tumwine LK, Riches C, Montague M, et al. Placental Plasmodium falciparum malaria infection: operational accuracy of HRP2 rapid diagnostic tests in a malaria endemic setting. Malar J. 2011;10:306.

393. Matangila J, Lufuluabo J, Ibalanky A, Inocêncio da Luz R, Lutumba P, Van Geertruyden J-P. Asymptomatic Plasmodium falciparum infection is associated with anaemia in pregnancy and can be more cost-effectively detected by rapid diagnostic test than by microscopy in Kinshasa, Democratic Republic of the Congo. Malar J. 2014;13(1):1-10.

394. Mayor A, Moro L, Aguilar R, Bardají A, Cisteró P, Serra-Casas E, et al. How Hidden Can Malaria Be in Pregnant Women? Diagnosis by Microscopy, Placental Histology, Polymerase Chain Reaction and Detection of Histidine-Rich Protein 2 in Plasma. Clin Infect Dis. 2012;54(11):1561-8.

395. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic Infection in Plasmodium falciparum-Endemic Populations: A Systematic Review and Meta-Analysis. J Infect Dis. 2009;200(10):1509-17.

396. Azikiwe CCA, Ifezulike CC, Siminialayi IM, Amazu LU, Enye JC, Nwakwunite OE. A comparative laboratory diagnosis of malaria: microscopy versus rapid diagnostic test kits. Asian Pac J Trop Biomed. 2012;2(4):307-10.

397. Batwala V, Magnussen P, Hansen KS, Nuwaha F. Cost-effectiveness of malaria microscopy and rapid diagnostic tests versus presumptive diagnosis: implications for malaria control in Uganda. Malar J. 2011;10:372.

398. Reyburn H, Mbakilwa H, Mwangi R, Mwerinde O, Olomi R, Drakeley C, et al. Rapid diagnostic tests compared with malaria microscopy for guiding outpatient treatment of febrile illness in Tanzania: randomised trial.BMJ.2007-02-22 23:05:50. 403 p.

399. Singer LM, Newman RD, Diarra A, Moran AC, Huber CS, Stennies G, et al. Evaluation of a malaria rapid diagnostic test for assessing the burden of malaria during pregnancy. Am J Trop Med Hyg. 2004;70(5):481-5.

400. Singh N, Saxena A, Awadhia SB, Shrivastava R, Singh MP. Evaluation of a rapid diagnostic test for assessing the burden of malaria at delivery in India. Am J Trop Med Hyg. 2005;73(5):855-8.

401. Desai M, Gutman J, L'Lanziva A, Otieno K, Juma E, Kariuki S, et al. Intermittent screening and treatment or intermittent preventive treatment with dihydroartemisinin-piperaquine versus intermittent preventive treatment with sulfadoxine-pyrimethamine for the control of malaria during pregnancy in western Kenya: an open-label, three-group, randomised controlled superiority trial. Lancet. 2015;386(10012):2507-19.

402. Capan-Melser M, Mombo Ngoma G, Akerey-Diop D, Basra A, Wurbel H, Groger M, et al. Evaluation of intermittent preventive treatment of malaria against group B Streptococcus colonization in pregnant women: a nested analysis of a randomized controlled clinical trial of

sulfadoxine/pyrimethamine versus mefloquine. The Journal of antimicrobial chemotherapy. 2015;70(6):1898-902.

403. Luntamo M, Rantala A-M, Meshnick SR, Cheung YB, Kulmala T, Maleta K, et al. The Effect of Monthly Sulfadoxine-Pyrimethamine, Alone or with Azithromycin, on PCR-Diagnosed Malaria at Delivery: A Randomized Controlled Trial. PLoS One. 2012;7(7):e41123.

404. Berhan Y, Berhan A. Antenatal Care as a Means of Increasing Birth in the Health Facility and Reducing Maternal Mortality: A Systematic Review. Ethiop J Health Sci. 2014;24(0 Suppl):93-104.

405. Hill J, Hoyt J, van Eijk AM, D'Mello-Guyett L, Ter Kuile FO, Steketee R, et al. Factors affecting the delivery, access, and use of interventions to prevent malaria in pregnancy in sub-Saharan Africa: a systematic review and meta-analysis. PLoS Med. 2013;10(7):e1001488.

406. Hill J, Kayentao K, Achieng F, Diarra S, Dellicour S, Diawara SI, et al. Access and Use of Interventions to Prevent and Treat Malaria among Pregnant Women in Kenya and Mali: A Qualitative Study. PLoS One. 2015;10(3):e0119848.

407. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. Lancet Infect Dis. 2007;7(2):105-17.

408. Rogerson SJ, Mwapasa V, Meshnick SR. Malaria in pregnancy: linking immunity and pathogenesis to prevention. Am J Trop Med Hyg. 2007;77(6 Suppl):14-22.

409. Sema M, Alemu A, Bayih AG, Getie S, Getnet G, Guelig D, et al. Evaluation of non-instrumented nucleic acid amplification by loop-mediated isothermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia. Malar J. 2015;14:44.

410. Polley SD, Mori Y, Watson J, Perkins MD, González IJ, Notomi T, et al. Mitochondrial DNA Targets Increase Sensitivity of Malaria Detection Using Loop-Mediated Isothermal Amplification. J Clin Microbiol. 2010;48(8):2866-71.

411. WHO. WHO Evidence Review Group on Malaria Diagnosis in Low Transmission Settings. Geneva, Switzerland: World Health Organization, 2014.

412. Snow RW. Global malaria eradication and the importance of Plasmodium falciparum epidemiology in Africa. BMC Med. 2015;13:23.

413. Liu J, Modrek S, Gosling RD, Feachem RGA. Malaria eradication: is it possible? Is it worth it? Should we do it? Lancet Glob Health. 2013;1(1):e2-e3.

414. Hay SI, Smith DL, Snow RW. Measuring malaria endemicity from intense to interrupted transmission. Lancet Infect Dis. 2008;8(6):369-78.

415. Nankabirwa JI, Wandera B, Amuge P, Kiwanuka N, Dorsey G, Rosenthal PJ, et al. Impact of Intermittent Preventive Treatment With Dihydroartemisinin-Piperaquine on Malaria in Ugandan Schoolchildren: A Randomized, Placebo-Controlled Trial. Clin Infect Dis. 2014;58(10):1404-12.

416. Ocan M, Bbosa GS, Waako P, Ogwal-Okeng J, Obua C. Factors predicting home storage of medicines in Northern Uganda. BMC Public Health. 2014;14:650.

417. Petersen EE, Rasmussen SA, Daniel KL, Yazdy MM, Honein MA. Prescription medication borrowing and sharing among women of reproductive age. J Womens Health (Larchmt). 2008;17(7):1073-80.

418. Planta MB. The role of poverty in antimicrobial resistance. J Am Board Fam Med. 2007;20(6):533-9.

419. Baud D, Greub G. Intracellular bacteria and adverse pregnancy outcomes. Clin Microbiol Infect. 2011;17(9):1312-22.

420. van den Broek NR, White SA, Goodall M, Ntonya C, Kayira E, Kafulafula G, et al. The APPLe Study: A Randomized, Community-Based, Placebo-Controlled Trial of Azithromycin for the Prevention of Preterm Birth, with Meta-Analysis. PLoS Med. 2009;6(12):e1000191.

421. Luntamo M, Kulmala T, Mbewe B, Cheung YB, Maleta K, Ashorn P. Effect of repeated treatment of pregnant women with sulfadoxine-pyrimethamine and azithromycin on preterm delivery in Malawi: a randomized controlled trial. Am J Trop Med Hyg. 2010;83(6):1212-20.

422. Unger HW, Ome-Kaius M, Wangnapi RA, Umbers AJ, Hanieh S, Suen CS, et al. Sulphadoxine-pyrimethamine plus azithromycin for the prevention of low birthweight in Papua New Guinea: a randomised controlled trial. BMC Med. 2015;13(1):9.

423. Unger HW, Aho C, Ome-Kaius M, Wangnapi RA, Umbers AJ, Jack W, et al. Impact of intermittent preventive treatment in pregnancy with azithromycin-containing regimens on maternal nasopharyngeal carriage and antibiotic sensitivity of Streptococcus pneumoniae, Haemophilus influenzae, and Staphylococcus aureus: a cross-sectional survey at delivery. J Clin Microbiol. 2015;53(4):1317-23.

424. Manyando C, Kayentao K, D'Alessandro U, Okafor HU, Juma E, Hamed K. A systematic review of the safety and efficacy of artemether-lumefantrine against uncomplicated Plasmodium falciparum malaria during pregnancy. Malar J. 2012;11:141.

425. Mosha D, Mazuguni F, Mrema S, Sevene E, Abdulla S, Genton B. Safety of artemether-lumefantrine exposure in first trimester of pregnancy: an observational cohort. Malar J. 2014;13:197.

426. Rulisa S, Kaligirwa N, Agaba S, Karema C, Mens PF, de Vries PJ. Pharmacovigilance of artemether-lumefantrine in pregnant women followed until delivery in Rwanda. Malar J. 2012;11:225.

427. Yeka A, Lameyre V, Afizi K, Fredrick M, Lukwago R, Kamya MR, et al. Efficacy and Safety of Fixed-Dose Artesunate-Amodiaquine vs. Artemether-Lumefantrine for Repeated Treatment of Uncomplicated Malaria in Ugandan Children. PLoS One. 2014;9(12):e113311.

428. Manning J, Vanachayangkul P, Lon C, Spring M, So M, Sea D, et al. Randomized, Double-Blind, Placebo-Controlled Clinical Trial of a Two-Day Regimen of Dihydroartemisinin-Piperaquine for Malaria Prevention Halted for Concern over Prolonged Corrected QT Interval. Antimicrob Agents Chemother. 2014;58(10):6056-67.

429. Darpo B, Ferber G, Siegl P, Laurijssens B, Macintyre F, Toovey S, et al. Evaluation of the QT effect of a combination of piperaquine and a novel anti-malarial drug candidate OZ439, for the treatment of uncomplicated malaria. Br J Clin Pharmacol. 2015:n/a-n/a.

430. Bassat Q, Mulenga M, Tinto H, Piola P, Borrmann S, Menendez C, et al. Dihydroartemisinin-piperaquine and artemether-lumefantrine for treating uncomplicated malaria in African children: a randomised, non-inferiority trial. PLoS One. 2009;4(11):e7871.

431. Valecha N, Phyo AP, Mayxay M, Newton PN, Krudsood S, Keomany S, et al. An open-label, randomised study of dihydroartemisinin-piperaquine versus artesunate-mefloquine for falciparum malaria in Asia. PLoS One. 2010;5(7):e11880.

432. Mpanga V, Maluwa, A. , Kafulafula, U. , Pindani, M. and Bultemeier, K. Comprehension of Risk Factors of Malaria during Pregnancy among Pregnant Women Attending Antenatal Care in Malawi. Open J Nurs. 2014;4:896-905.

433. Kiwuwa MS, Mufubenga P. Use of antenatal care, maternity services, intermittent presumptive treatment and insecticide treated bed nets by pregnant women in Luwero district, Uganda. Malar J. 2008;7:44.

434. Wumba RD, Zanga J, Aloni MN, Mbanzulu K, Kahindo A, Mandina MN, et al. Interactions between malaria and HIV infections in pregnant women: a first report of the magnitude, clinical and laboratory features, and predictive factors in Kinshasa, the Democratic Republic of Congo. Malar J. 2015;14:82.

435. Turner AN, Tabbah S, Mwapasa V, Rogerson SJ, Meshnick SR, Ackerman WEt, et al. Severity of maternal HIV-1 disease is associated with adverse birth outcomes in Malawian women: a cohort study. J Acquir Immune Defic Syndr. 2013;64(4):392-9.

436. Denoeud-Ndam L, Zannou DM, Fourcade C, Taron-Brocard C, Porcher R, Atadokpede F, et al. Cotrimoxazole prophylaxis versus mefloquine intermittent preventive treatment to prevent malaria

in HIV-infected pregnant women: two randomized controlled trials. J Acquir Immune Defic Syndr. 2014;65(2):198-206.

437. Kapito-Tembo A, Meshnick SR, van Hensbroek MB, Phiri K, Fitzgerald M, Mwapasa V. Marked Reduction in Prevalence of Malaria Parasitemia and Anemia in HIV-Infected Pregnant Women Taking Cotrimoxazole With Or Without Sulfadoxine-Pyrimethamine Intermittent Preventive Therapy during Pregnancy in Malawi. J Infect Dis. 2011.

438. Gimnig JE, MacArthur JR, M'bang'ombe M, Kramer MH, Chizani N, Stern RS, et al. Severe cutaneous reactions to sulfadoxine-purimethamine and trimethoprim-sulphamethoxazole in Blantyre district, Malawi. Am J Trop Med Hyg. 2006;74(5):738-43.

439. Sevene E, Gonzalez R, Menendez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. Expert Opin Pharmacother. 2010;11(8):1277-93.

440. Ward SA, Sevene EJ, Hastings IM, Nosten F, McGready R. Antimalarial drugs and pregnancy: safety, pharmacokinetics, and pharmacovigilance. Lancet Infect Dis. 2007;7(2):136-44.

441. Manyando C, Njunju EM, D'Alessandro U, Van geertruyden J-P. Safety and Efficacy of Co-Trimoxazole for Treatment and Prevention of Plasmodium falciparum Malaria: A Systematic Review. PLoS One. 2013;8(2):e56916.

442. Iyer JK, Milhous WK, Cortese JF, Kublin JG, Plowe CV. Plasmodium falciparum cross-resistance between trimethoprim and pyrimethamine. Lancet. 2001;358(9287):1066-7.

443. Hamel MJ, Greene C, Chiller T, Ouma P, Polyak C, Otieno K, et al. Does cotrimoxazole prophylaxis for the prevention of HIV-associated opportunistic infections select for resistant pathogens in Kenyan adults? Am J Trop Med Hyg. 2008;79(3):320-30.

444. Malamba SS, Mermin J, Reingold A, Lule JR, Downing R, Ransom R, et al. Effect of cotrimoxazole prophylaxis taken by human immunodeficiency virus (HIV)-infected persons on the selection of sulfadoxine-pyrimethamine-resistant malaria parasites among HIV-uninfected household members. Am J Trop Med Hyg. 2006;75(3):375-80.

445. Klement E, Pitche P, Kendjo E, Singo A, D'Almeida S, Akouete F, et al. Effectiveness of co-trimoxazole to prevent Plasmodium falciparum malaria in HIV-positive pregnant women in sub-Saharan Africa: an open-label, randomized controlled trial. Clin Infect Dis. 2014;58(5):651-9.

446. Mbeye NM, ter Kuile FO, Davies MA, Phiri KS, Egger M, Wandeler G. Cotrimoxazole prophylactic treatment prevents malaria in children in sub-Saharan Africa: systematic review and meta-analysis. Trop Med Int Health. 2014;19(9):1057-67.

447. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and preventing HIV infection. Geneva, Switzerland: World Health Organization, 2013.

448. Capan M, Mombo-Ngoma G, Makristathis A, Ramharter M. Anti-bacterial activity of intermittent preventive treatment of malaria in pregnancy: comparative in vitro study of sulphadoxine-pyrimethamine, mefloquine, and azithromycin. Malar J. 2010;9(1):303.

9 ANNEXES

9.1 Annex 1: STARD checklist for the reporting of studies of diagnostic accuracy

(Version January 2003)

Section and Topic	Item #		On page #
TITLE/ABSTRACT/ KEYWORDS	1	Identify the article as a study of diagnostic accuracy (recommend MeSH heading 'sensitivity and specificity').	
INTRODUCTION	2	State the research questions or study aims, such as estimating diagnostic accuracy or comparing accuracy between tests or across participant groups.	
METHODS			
Participants	3	The study population: The inclusion and exclusion criteria, setting and locations where data were collected.	
	4	Participant recruitment: Was recruitment based on presenting symptoms, results from previous tests, or the fact that the participants had received the index tests or the reference standard?	
	5	Participant sampling: Was the study population a consecutive series of participants defined by the selection criteria in item 3 and 4? If not, specify how participants were further selected.	
	6	Data collection: Was data collection planned before the index test and reference standard were performed (prospective study) or after (retrospective study)?	
Test methods	7	The reference standard and its rationale.	
	8	Technical specifications of material and methods involved including how and when measurements were taken, and/or cite references for index tests and reference standard.	
	9	Definition of and rationale for the units, cut-offs and/or categories of the results of the index tests and the reference standard.	
	10	The number, training and expertise of the persons executing and reading the index tests and the reference standard.	
	11	Whether or not the readers of the index tests and reference standard were blind (masked) to the results of the other test and describe any other clinical information available to the readers.	
Statistical methods	12	Methods for calculating or comparing measures of diagnostic accuracy, and the statistical methods used to quantify uncertainty (e.g. 95% confidence intervals).	
	13	Methods for calculating test reproducibility, if done.	
RESULTS			
Participants	14	When study was performed, including beginning and end dates of recruitment.	
	15	Clinical and demographic characteristics of the study population (at least information on age, gender, spectrum of presenting symptoms).	
	16	The number of participants satisfying the criteria for inclusion who did or did not undergo the index tests and/or the reference standard; describe why participants failed to undergo either test (a flow diagram is strongly recommended).	
Test results	17	Time-interval between the index tests and the reference standard, and any treatment administered in between.	
	18	Distribution of severity of disease (define criteria) in those with the target condition; other diagnoses in participants without the target condition.	
	19	A cross tabulation of the results of the index tests (including indeterminate and missing results) by the results of the reference standard; for continuous results, the distribution of the test results by the results of the reference standard.	

	20	Any adverse events from performing the index tests or the reference standard.	
Estimates	21	Estimates of diagnostic accuracy and measures of statistical uncertainty (e.g. 95% confidence intervals).	
	22	How indeterminate results, missing data and outliers of the index tests were handled.	
	23	Estimates of variability of diagnostic accuracy between subgroups of participants, readers or centers, if done.	
	24	Estimates of test reproducibility, if done.	
DISCUSSION	25	Discuss the clinical applicability of the study findings.	

9.2 Annex 2: Patient information sheet (English version only)

The University Of Malawi College Of Medicine and the Liverpool School of Tropical Medicine are jointly doing a research study. We invite you to take part in this study. Before you decide whether to take part, it is important for you to understand why the study is being done and what it will involve. Please take the time to read the following information carefully. Ask us if there is anything that is not clear and if you would like more information.

What is the purpose of the study?

Malaria is a common cause of anaemia (lack of blood) in pregnancy and can cause the baby to be born small and weak. Sometimes you can have malaria without feeling sick. To help prevent malaria in pregnancy, you would usually be given three tablets of sulphadoxine-pyrimethamine (SP or Fansidar) three or four times during pregnancy when you visit the clinic after you first feel the baby move. This strategy is called IPTp-SP, which stands for **I**ntermittent **P**reventive **T**reatment in **p**regnancy with **S**ulphadoxine-**P**yrimethamine.

Malaria parasites are now becoming resistant to SP, meaning that the drug may not work as well as it used to. We want to evaluate a new strategy for control of malaria in pregnancy called ISTp. ISTp stands for **I**ntermittent **S**creening and **T**reatment in **p**regnancy, and consists of testing women for malaria three or four times during pregnancy, and, if they have malaria, treating them with a drug called dihydroartemisinin-piperaquine (DHA-PQ), which is used to cure malaria. We want to compare the new strategy (ISTp) with IPTp-SP given three or four times.

Why have I been chosen?

We need women who are between 16 and 28 weeks pregnant, and who would normally be scheduled to receive IPTp-SP, to take part in this study. If you are less than 16 weeks pregnant, we would like you to join the study after you are 16 weeks pregnant, when you next come to the clinic.

What will happen if I want to take part?

If you think you would like to be in this study, we will first invite you to be screened, to see if you are suitable. If you agree we will first take a small amount of blood from your arm (about a teaspoon) and test it for anaemia and HIV, unless you have already had an HIV test in this pregnancy. Either then or later (perhaps after you join the study) we will do an ultrasound scan of your baby. An ultra-sound scan is a test which shows a moving picture of the baby inside you. You will be asked to lie down, a cool gel will be spread over your belly and a small machine will be moved gently over it. We will use the ultrasound picture to know when your baby is due to be born. Ultra-sound scans are completely painless and harmless to you and the baby. They are very common in Europe and America.

If the blood tests show that you are suitable for this study, and you agree to take part, we will ask you some questions about your health. We will do a physical examination, measure your weight, and copy some information from your health passport and from the lab books. We will use the rest of blood sample that we have already taken to find out whether you have malaria, and the nurse will also do all the other routine tests that pregnant women usually have, unless you have already had these tests. If you consent, we will also send some of this blood sample abroad for special tests of your body' resistance against the malaria parasite (immunity) and the resistance of the parasite. We will try not to prick your arm again at this appointment. The examination and tests will take about ten minute's altogether.

You will then open an envelope with a piece of paper inside which describes which treatment you will get. The treatment you get is decided by chance, like flipping a coin. It will be either:

- ***Group 1 - ISTp***

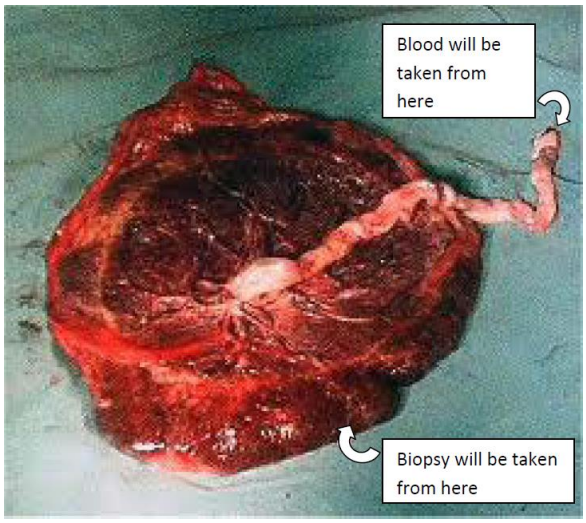
If you are in this group, we will test the blood sample for malaria using a rapid diagnostic test. The test results will take about 15 minutes. If the results show that you have malaria, we will give you DHA-PQ tablets to take in the clinic and some more tablets to take at home on the following day and the day after that. If you are given DHA-PQ, a fieldworker may visit you at home on the second day after your appointment to ask whether you have taken the tablets and whether you had any problems with them. You will only receive DHA-PQ if you have malaria.

Or

- ***Group 2 -*** **IPTp-SP** (current standard of care)

If you are in this group you will be given three tablets of SP to take at the clinic. You will not be tested for malaria.

You will then be asked to return to the study clinic around two or three more times, when you will be given the same treatment that you received on your first visit. If you are tested for malaria at one of these visits we will take only a tiny amount of blood from a finger prick. At one of these visits, we will ask you for an additional blood sample, taken from a finger, which we will test for malaria at the end of the study. We will also use this blood sample to test you for anaemia. If we test you and find that you have anaemia, we will treat your anaemia at this visit.



When you come for your clinic visits we will give you enough money for transport to and from the clinic and, if you miss lunch, for a light meal. You will also receive all other health care that pregnant women attending the clinic would usually receive.

We would like you to give birth to your baby in the maternity ward of the clinic so that we can check how you and the baby are doing. After your baby is born we will check the placenta for malaria (see picture).

We will take a blood sample from your arm and test it for malaria, your level of immunity to malaria, and anaemia. We will measure your baby's weight and give the baby a health check. If you want to give birth to your baby at home, we would like you to inform us as soon as possible when the baby is born, so we can visit you at home and collect information for the study.

We would like to see you again with your baby when baby is seven days old and again when they are about six to eight weeks old, to check that you and your baby are still doing well. You will not need to make an extra journey to the clinic, as we will see you when you bring your baby for their routine vaccinations.

If you are in the study, we would prefer that you do not take any medicine that is not provided by the clinic. If you feel unwell during the study you can come to the clinic. You will be seen by a qualified member of staff free of charge and will be given any treatment that you need.

The information collected will be used by staff involved in the study and by scientists in Malawi and abroad. It will be kept confidential. Names and addresses will not appear on any of the study reports.

What if I don't want to be in the study?

If you decide not to be in the study, you will receive all the usual health care that pregnant women attending the clinic usually receive. This will include getting three tablets of SP when you visit the clinic at least two times during pregnancy after you first feel the baby move, and again several times later in your pregnancy. You can change your mind and withdraw from the study at any time, and you do not have to give a reason if you do not want to.

What are the possible disadvantages and risks of taking part?

DHA-PQ is a very effective treatment for malaria. It has already been tested in pregnant women after 13 weeks of pregnancy and it does not seem to cause any harm to women or their babies. However, it is still quite a new drug and we cannot be completely sure that it will not have any side-effects. If you appear to suffer bad side-effects during the study, we will stop your treatment with DHA-PQ. All babies will be given a thorough health check and any problems will be recorded. The study will be stopped if we suspect that DHA-PQ is causing health problems in the babies

SP has been widely used during pregnancy, is very safe, and is given to all pregnant women who attend clinics in Malawi. You will also be given SP if you do not take part in the study.

If do you become ill after taking DHA-PQ or SP, and we think it might be because of the drug, we may stop your treatment (not give you the same drug again) but if this happens we would still like you to continue in the study.

When we take blood, you may get a small bruise or mild pain on the finger or arm where the blood is taken. There is also a very small chance of infection; the chance is very small because we always use clean materials.

The study will require you to make more trips to the clinic than usual, which may be inconvenient. However, we will give you money to pay for the travel costs.

Thank you very much for your time. Would you like to be screened to join the study?

9.3 Annex 3: Study informed consent forms (English version only)

9.3.1 Consent for screening

Screening will involve taking a small sample of blood from your arm for testing, and possibly also an ultrasound scan.

If you agree to be screened, this does not mean that you have to agree to be in the study, you can change your mind at any time.

The above study has been explained to me and I agree to be screened to see if I am suitable to be in the study.

Participant's statement: sign or thumbprint on the appropriate line. If the participant does not wish to consent, form should not be signed.

The above has been explained to me	I agree to be part of this study	
Participant's name:	Participant 's signature:	Date: (day/month/year)

Witness' statement (optional, except for illiterate participants):

The above consent was explained and the woman agreed to be screened:

Witness' name	Witness' signature	Date: (day/month/year)

Investigator's name:	Investigator 's signature:	Date: (day/month/year)

9.3.2 Consent for inclusion in the study

The above study has been explained to me and I agree to join.

- I have been told about the risks of and benefits of being in the study.
- I have been told that it is up to me if I want to join the study and that I can leave the study any time I want without any consequences for me and my baby.
- I agree to have a home visit by the study staff if I am not able to come to the study clinic.

Participant's statement: sign or thumbprint on the appropriate line. If the participant does not wish to consent, form should not be signed.

The above has been explained to me	I agree to be part of this study	
Participant's name:	Participant 's signature:	Date: (day/month/year)

Witness' statement (optional, except for illiterate participants):

The above consent was explained and the woman agreed to join the study:

Witness' name	Witness' signature	Date: (day/month/year)

Investigator's name:	Investigator 's signature:	Date: (day/month/year)

Contacts

If you later have questions or concerns about your participation in this study, you may speak with one of our staff. If you have any questions about the study, or you feel your child has been harmed, please contact Dr. Linda Kalilani-Phiri at the College of Medicine, Private Bag 360, Blantyre 3 or at phone number 01919776**.** If you have any questions about your rights as a study participant, or if you want to talk about the study with someone who is not part of this research project, please contact Prof Mfutso-Bengo at the College of Medicine, Private Bag 360, Blantyre 3 or phone number 01877291**.**

9.3.3 Consent for transportation and storage of blood samples

We would like to send part of the blood sample abroad for special tests of your body' resistance against the malaria parasite (immunity) and the resistance of the parasite. We do not have the equipment to be able to do these tests in Malawi at present.

We would also like to store a part of the blood sample collected in this research in case there are additional tests that we wish to perform after the study is over. The sample will be sent to the USA and stored there. We will not store your samples without your permission.

If you allow us to transport and store your samples, you may change your mind and withdraw up to one month after you complete the study. If you would like your samples to be removed from storage, you may contact Dr. _____ at phone number _____.

One month after you complete the study, we will remove your name from the blood samples sent for storage. After your name is removed, we will not be able to take your samples out of storage if you change your mind. Also, we will not be able to report any future test results to you.

You can be in the study even if you do not want blood samples stored.

Please check one of the boxes below to indicate whether you do or do not allow us to store your blood.

☐ NO, I wish my blood samples to be destroyed immediately.

☐ YES, I give permission for my blood samples to be stored anonymously

Participant's statement: sign or thumbprint on the appropriate line. If the participant does not wish to consent, form should not be signed.

The above has been explained to me	I agree to be part of this study	
Participant's name:	Participant 's signature:	Date: (day/month/year)

Witness' statement (optional, except for illiterate participants):

The above consent was explained and the woman agreed to join the study:

Witness' name	Witness' signature	Date: (day/month/year)

Investigator's name:	Investigator 's signature:	Date: (day/month/year)